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PA ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HUGHES, E., John, L.
Davies Collison Cave
Level 3
303 Coronation Drive
Milton, Queensland 4064
AUSTRALIE

Date of mailing (day/month/year)
21 June 2000 (21.06.00)

Applicant's or agent's file reference
2211601/EJH

IMPORTANT NOTIFICATION

International application No.
PCT/AU99/01004

International filing date (day/month/year)
12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
ANALYTICA LTD
194-198 St Kilda Road
St Kilda, Victoria 3182
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
ANALYTICA LTD
Suite 201
566 St Kilda Road
St Kilda, VIC 3182
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
F. Baechler
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 June 2000 (21.06.00)	
International application No. PCT/AU99/01004	**Applicant's or agent's file reference** 2211601/EJH
International filing date (day/month/year) 12 November 1999 (12.11.99)	**Priority date** (day/month/year) 12 November 1998 (12.11.98)
Applicant TSENG, Albert, Peng, Sheng et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 June 2000 (02.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

AU9901004

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2211601	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. PCT/AU99/01004	International filing date *(day/month/year)* 12 November 1999	Priority Date *(day/month/year)* 12 November 1998
International Patent Classification (IPC) or national classification and IPC Int. Cl. [7] A61K 31/70		
Applicant ANALYTICA LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 2 June 2000	Date of completion of the report 26 September 2000
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **BERNARD NUTT** Telephone No. (02) 6283 2491

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
P[redacted]U99/01004

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	7,8	YES
	Claims	1-6, 9-13	NO
Inventive step (IS)	Claims	7,8	YES
	Claims	1-6, 9-13	NO
Industrial applicability (IA)	Claims	1-13	YES
	Claims	-	NO

2. Citations and explanations (Rule 70.7)

D1: WO 91/09603 (The Wellcome Foundation Limited) 11 July 1991

D2: US 5663059 (Hawkins P.R. et al) 2 September 1997

D3: WO 98/10776 (Shanahan-Prendergast, E) 19 March 1998

D4: US 5811520 (Hawkins P.R. et al) 22 September 1998

D5: WO 97/35588 (UAB Research Foundation) 2 October 1997

Claims 1-6,11-13 are not considered to be novel or inventive. Citations 1-5 disclose phospholipase inhibitors, for the treatment of cancer. All of the citations fall within the scope of claims 1-6. Claims 11 and 13 are "not limited" by the PLA inhibitors of the invention and claims 9 and 12 only need to have amino acid sequences "substantially" as set forth in the PLA inhibitors of the invention. There is no guidance in the specification as to what "substantially" means in this context.

Claims 7 and 8 are considered novel and inventive as PLA inhibitors from Australian tiger snake *Notechis scutatus* or *Notechis ater* are not mentioned in the prior art documents.

Claims 1-10,12 relate to methods of treatment of the human or animal body, and as such are not required to be reported upon under Article 34(4)(a)(i). These claims do however form part of this report as they do not contravene Australian law.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/01004

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K AND KEYWORDS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent, Chemical abstracts. Keywords: phospholipase inhibitor, cancer, tumour, metastatis, notechis, scutatus, ater, snake

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91/09603 A (THE WELLCOME FOUNDATION LIMITED) 11 July 1991 (see whole document)	1 - 6,9 - 13
X	US 5663059 A (Hawkins P.R. et al) 2 September 1997 (see whole document)	1 - 6, 9 - 13
X	WO 98/10776 A (Shanahan-Prendergast, E) 19 March 1998 (see whole document)	1 - 6, 9 - 13

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1999	05 JAN 2000

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No.: (02) 6285 3929	BERNARD NUTT Telephone No.: (02) 6283 2491

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/01004

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5811520 A (Hawkins, P.R. et al) 22 September 1998	1 - 6, 9 - 13
X	WO 97/35588 A (UAB RESEARCH FOUNDATION) 2 October 1997	1 - 6, 9 - 13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU99/01004

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	91/09603	EP	438921	JP	4504729	US	5330977
		IT	1238356				
US	5663059	AU	31202/97	WO	9744454	EP	904372
WO	98/10776	AU	41323/97				
US	5811520	AU	31202/97	WO	9744454	EP	904372
WO	97/3558	AU	25456/97				

END OF ANNEX

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2211601	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. **PCT/AU99/01004**	International filing date *(day/month/year)* 12 November 1999	(Earliest) Priority Date *(day/month/year)* 12 November 1998

Applicant

1. ANALYTICA LTD et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **4** sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

Phospholipase inhibitors for the treatment of cancer

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/01004

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K AND KEYWORDS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent, Chemical abstracts. Keywords: phospholipase inhibitor, cancer, tumour, metastatis, notechis, scutatus, ater, snake

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91/09603 A (THE WELLCOME FOUNDATION LIMITED) 11 July 1991 (see whole document)	1 - 6,9 - 13
X	US 5663059 A (Hawkins P.R. et al) 2 September 1997 (see whole document)	1 - 6, 9 - 13
X	WO 98/10776 A (Shanahan-Prendergast, E) 19 March 1998 (see whole document)	1 - 6, 9 - 13

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 December 1999

Date of mailing of the international search report
05 JAN 2000

Name and mailing address of the ISA/AU

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PO BOX 200
WODEN ACT 2606 AUSTRALIA
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Facsimile No.: (02) 6285 3929

Authorized officer

BERNARD NUTT
Telephone No.: (02) 6283 2491

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 249/06, C12N 5/08, A61K 31/41

A1

(11) International Publication Number: WO 96/40657

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/10221

(22) International Filing Date: 6 June 1996 (06.06.96)

(30) Priority Data:

08/486,203	7 June 1995 (07.06.95)	US
08/658,796	5 June 1996 (05.06.96)	US

(71) Applicant: THE GEORGE WASHINGTON UNIVERSITY [US/US]; Suite 712, 2300 Eye Street, N.W., Washington, DC 20037 (US).

(72) Inventors: PATIERNO, Stephen, R.; 2906 Brook Drive, Falls Church, VA 22042 (US). MANYAK, Michael, J.; 2322 Blaine Drive, Chevy Chase, MD 20815 (US).

(74) Agent: NATH, Gary, M.; Nath & Associates, Suite 750, 1835 K Street, N.W., Washington, DC 20006-1203 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: INHIBITORS OF PHOSPHOLIPASE A_2

(57) Abstract

The invention relates to methods for identifying prostatic intraepithelial neoplasia, methods for determining metastatic potential of tumors, and to methods and compositions for inhibiting or preventing metastasis of cancers. In one aspect, the invention provides a method to determine metastatic potential of tumors, particularly prostatic tumors. In another aspect, the invention provides a method of identifying prostate cancer associated conditions, particularly prostatic intraepithelial neoplasia. In these regards, the invention relates to determining protein or mRNA of effectors of arachidonic acid release, particularly uteroglobin protein or mRNA, to identify intermediate conditions such as PIN or to gauge metastatic potential of prostatic tumors. The invention also relates to methods and compositions that prevent or inhibit metastasis of cancers. In this regard, the invention particularly relates to methods and compositions that inhibit arachidonic acid, those that inhibit phospholipase A_2. More particularly in this regard, the invention relates to uteroglobin or muteins, peptide analogs or mimetics of uteroglobin and lipocortins or muteins, peptide analogs, or mimetics of lipocortins that inhibit metastasis. Especially it relates to methods and compositions in which uteroglobins, particularly human uteroglobins, inhibit or prevent metastasis of cancer, particularly prostatic cancer.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
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INHIBITORS OF PHOSPHOLIPASE A_2

(This application is a continuation-in-part of U.S patent application serial number 08/486,203, filed June 7, 1995, which is a continuation-in-part of U.S. patent application serial number 08/400,084, filed on March 7, 1995, the entirety of both of which are incorporated by reference herein.)

The present invention relates to methods and compositions that provide for the diagnosis and treatment of prostatic intraepithelial neoplasia. A particular aspect of the invention relates to methods and compositions containing compounds which inhibit phospholipase A_2, particularly those that contain uteroglobin, uteroglobin muteins, uteroglobin mimetics, peptide analogs of uteroglobins, lipocortins, lipocortin muteins and peptide analogs of lipocortins. Further compositions of the invention include other types of active ingredients in combination with those described above.

The present invention also relates to methods for gauging the metastatic potential of tumors of epithelial cell origin by determining an effector of arachidonic acid release in cells of a tumor-containing tissue. This aspect of the invention particularly relates to determining uteroglobin protein or mRNA in cells of a biopsy sample to determine metastatic potential of a prostatic tumor.

The present invention further relates to methods and compositions that prevent or inhibit metastases of cancers of epithelial cell origin, especially human prostate cancers. A particular aspect of the invention relates to methods and compositions that inhibit arachidonic acid release in cells of these cancers and inhibit or prevent metastasis. In one aspect in this regard, the invention particularly relates to methods and compositions that inhibit phospholipase A_2 that mediates arachidonic acid release in the cancer cells. Compositions of the invention also particularly include those that contain uteroglobin, uteroglobin muteins, peptide analogs of uteroglobins, lipocortins, lipocortin muteins and peptide analogs of lipocortins that inhibit arachidonic acid release by cancer cells. Further useful in this regard are mimetic compounds, particularly uteroglobin and lipocortin mimetics. In this regard, the invention relates especially to compositions that contain mimetics of uteroglobin, particularly of human uteroglobin. Further compositions of the invention include other types of active ingredients in combination with those that inhibit arachidonic acid release.

The invention also particularly relates to methods to prevent or inhibit metastases of human cancers of epithelial cell origin by administering the foregoing compositions. Especially in this regard the

invention relates to methods using human uteroglobin to inhibit or prevent metastasis of human prostate cancers. Further, this aspect of the invention may be accomplished by genetic therapy.

Methods and compositions of the invention may be used by themselves and with other treatment modalities.

BACKGROUND OF THE INVENTION

Cancers develop from uncontrolled multiplication of cells. All cancers are life threatening. Even when cancer does not result in death, it is permanently debilitating, not only to the patient, but also to family, friends and co-workers. Too often, moreover, cancers prove fatal. The personal and public loss from this cluster of diseases, which cause a significant fraction of all premature deaths, is beyond estimation.

Although effective treatment modalities have been developed in a few cases, many cancers remain refractory to currently available therapies. Particularly difficult to treat are metastatic cancers. These cancers pose the highest risk to patients and, for optimal prognosis, often must be treated by aggressive methods that present increased risks of deleterious side-effects. Therefore, there

is a great need for methods that accurately distinguish those tumors that are likely to metastasize from those that are unlikely to do so. Furthermore, methods for treating metastatic cancers often are inadequate, and there also is a clear need for improved anti-metastatic agents and methods to treat metastatic cancers.

Similarly, there is a great need for methods that accurately identify cells that are associated with prostate cancer, such as those found in prostatic intraepithelial neoplasia (PIN). Current diagnostic methods are inadequate to differentiate between PIN and normal cells. Thus, there is a clear need for improved early detection of PIN which may allow for early diagnosis, prognosis, and treatment of cancer.

Metastatic cancers originate from a primary tumor. Metastasis of the primary tumor produces secondary tumors and disseminated cancer. It is well known that both primary and secondary tumors shed large numbers of cells. The shed cells can spread through the body. For instance, a primary tumor may damage the surrounding lymph or circulatory vessels, allowing entry of shed cells into the lymph or circulatory systems, and hastening their spread in the body. Moreover, shedding of cells by cancerous tumors increases during surgery and radiotherapy.

Most shed cells do not form new tumors. To do so such cells must surmount a series of physical and

physiological barriers. In fact, a series of distinct events must occur for metastasis to occur. The primary tumor physically must (i) invade interstitial space of the primary tissue. In particular, it must (ii) penetrate the basement membrane of the tissue. For most metastases the tumor must damage the endothelial cell wall of lymphatic or vascular vessels to provide access to shed cells. Cells that enter the lymph or blood must (iii) survive hemodynamic stress and host defenses in the circulation and, furthermore, (iv) the cells must lodge at a new site in the circulatory system, a process that apparently involves aggregated platelets. A cell then must (v) extravasate out of the vessel into the interstitial space. Finally, it must (vi) invade the interstitial space of the secondary organ and proliferate in the new location. Although the process of metastasis is physiologically complex, the overall pattern of metastasis is general to many types of cancers.

The metastatic process also clearly involves complex intracellular mechanisms that alter cancerous cells and their interactions with surrounding cells and tissues. For instance, cancerous cells are characterized by aberrant expression of adhesion proteins, enzymes that degrade matrix components, autocrine factors, ligand-responsive receptors, factors of angiogenesis and prostaglandins, to name a few. In particular, the signaling pathways that

initiate tumor cell migration are among the least understood aspects of invasion and metastasis. Currently, it is thought that proliferation of many cancerous cells depends upon specific ligand-receptor interactions. Thus far, however, it has not been possible to use this paradigm, or other concepts of the underlying mechanisms of metastasis, to develop a therapy that prevents or effectively inhibits metastasis of metastatic cancers.

The complexity of the processes involved in metastasis, and the lack of understanding of underlying molecular mechanisms, have made it particularly difficult, in some cases, to distinguish tumors that are likely to metastasize from those that are unlikely to do so. The inability to discern the metastatic potential of tumors precludes accurate prognosis and leads, inevitably, to the therapeutic intervention that either is too aggressive or insufficiently aggressive. Furthermore, for all types of cancers it has been difficult or impossible, thus far, to develop treatments that inhibit or prevent the spread of metastatic tumors. Clearly, there remains a great need for methods to accurately determine the metastatic potential of tumors and for effective anti-metastatic compositions and methods.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide methods and compositions for differentiating PIN from normal prostate epithelia.

It is also an object of the present invention to provide methods and compositions for early detection of prostate cancer and cells associated with prostate cancer.

It is another object of the present invention to provide methods for inhibiting or preventing metastasis.

It is another object of the present invention to provide compositions for inhibiting or preventing metastasis.

In accomplishing the foregoing objects, there has been provided, in accordance with one aspect of the present invention, a method for identifying prostatic intraepithelial neoplasia, comprising the step of administering to an organism suffering from a cancer of epithelial cell origin a compound that inhibits arachidonic acid release by cells of the cancer by a route and in an amount and manner effective to identify the prostatic intraepithelial neoplasia.

Another method of the present invention is directed to a diagnostic kit for the detection of prostatic intraepithelial neoplasia in a biopsy sample, the kit comprising: a first reagent that binds specifically to an effector of arachidonic acid

release in cells in a biopsy sample prepared for determination of the effector, and a second reagent for detectably labelling the primary binding reagent bound specifically to cells in the biopsy sample, wherein the determination of the effector is diagnostic of prostatic intraepithelial neoplasia.

In certain preferred embodiments of the kits of the invention, the effector is an inhibitor of PLA_2, among which uteroglobin is particularly preferred.

In certain further preferred embodiments of the this aspect of the invention, the first reagent is an antibody. In these embodiments, the determination would occur where uteroglobin-antibody staining would indicate normal prostate epithelia if strong staining occurred, prostatic intraepithelial neoplasia if weak staining occurred, and cancer if no signal occurred.

Additional preferred embodiments of this aspect of the invention are those in which the first reagent is a hybridization probe. In certain preferred embodiments of this aspect of the invention, the effector is an inhibitor of PLA_2, among which uteroglobin is particularly preferred.

Another preferred embodiment of the present invention is directed to a method for preventing or inhibiting metastasis of a cancer of epithelial cell origin, comprising the step of administering to an organism suffering from a cancer of epithelial cell origin a compound that inhibits arachidonic acid

release by cells of the cancer by a route and in an amount effective to inhibit or prevent metastasis of the tumor.

In a preferred embodiment of an aspect of the invention in this regard, the compound is an inhibitor of phospholipase A_2 or cyclooxygenase. Particularly, phospholipase A_2 inhibitors are preferred.

In certain particularly preferred embodiments of this aspect of the invention, the compound is a uteroglobin, a mutein of a uteroglobin, a peptide analog of a uteroglobin, a mimetic of uteroglobin, a lipocortin, a mutein of a lipocortin, a peptide analog of a lipocortin or a mimetic of lipocortin. Especially highly preferred in this regard are methods wherein the compound is a uteroglobin, a mutein of a uteroglobin, a peptide analog of a uteroglobin or a mimetic of uteroglobin. Uteroglobin is preferred and human uteroglobin is particularly highly preferred in this regard.

Also there is provided in accordance with this aspect of the invention certain preferred embodiments in which the compound is a small molecule drug that is a nonsteroidal anti-inflammatory agent. Among these agents inhibitors of phospholipase A_2 and cyclooxygenase are preferred. Particularly preferred are mepacrine and indomethacin.

In another regard, preferred embodiments of the present method are those used to treat a cancer of the prostate gland in a human patient.

In further preferred embodiments, the method is used in conjunction with another treatment. In this regard, preferred treatments include surgical intervention, radiation therapy, hormonal therapy, immunotherapy, chemotherapy, cryotherapy or gene therapy.

In accordance with another aspect of the present invention, there has been provided a pharmaceutical composition for inhibiting or preventing metastasis of a cancer of epithelial cell origin, comprising: (i) a compound that inhibits arachidonic acid release by cells of a tumor of epithelial cell origin effective to inhibit or prevent metastasis of the tumor in an organism and (ii) a carrier for effective the therapeutic administration of the compound to the organism.

In certain preferred embodiments of the invention the compound is an inhibitor of phospholipase A_2 or cyclooxygenase. In this regard, inhibitors of phospholipase A_2 are preferred. In certain particularly preferred embodiments the compound is a uteroglobin, a mutein of a uteroglobin, a peptide analog of a uteroglobin, a mimetic of uteroglobin, a lipocortin, a mutein of a lipocortin a peptide analog of a lipocortin or a mimetic of lipocortin. In

especially preferred embodiments in this regard the compound is a uteroglobin, a mutein of a uteroglobin or a peptide analog of a uteroglobin. Among these, uteroglobins are very highly preferred, and human uteroglobins are among the most highly preferred compounds of the present invention.

Also there is provided in accordance with this aspect of the invention certain preferred embodiments in which the compound is a small molecule drug that is a nonsteroidal anti-inflammatory agent. Among these agents inhibitors of phospholipase A_2 and cyclooxygenase are preferred. Particularly preferred are mepacrine and indomethacin.

In accordance with another aspect of the invention there has been provided a method for determining metastatic potential of a tumors, particularly those of epithelial cell origin. In certain preferred embodiments of this aspect of the invention there has been provided a method for determining the metastatic potential of tumors of epithelial cell origin comprising the steps of (A) determining an effector of arachidonic acid release in cells in a biopsy sample of a tumor; (B) comparing effector in tumor cells in the biopsy sample with effector in fiduciary cells, and (C) determining metastatic potential, wherein effector in the tumor cells characteristic of normal fiduciary cells or characteristic of fiduciary cells of benign tumors

indicates low metastatic potential and effector in the tumor cells characteristic of fiduciary cells of metastatic tumors indicates high metastatic potential.

In some preferred embodiments of this aspect of the invention the effector is an inhibitor of PLA_2. In particularly preferred embodiments in this regard, the effector is uteroglobin.

In certain preferred embodiments the effector is determined by assaying the effector protein in cells of the tumor. In particularly preferred embodiments in this regard, the effector is an inhibitor of PLA_2. Especially preferred is uteroglobin. In particularly preferred embodiments in this regard the tumor is a prostatic tumor and the inhibitor is uteroglobin.

In another aspect of the invention, preferred embodiments of the invention provide methods for determining metastatic potential in which a protein is assayed by immunocytochemistry. In certain preferred embodiments of this type, the effector is an inhibitor of PLA_2. Particularly preferred in embodiments of the invention in this regard is uteroglobin. In particularly preferred embodiments in this regard the tumor is a prostatic tumor and the inhibitor is uteroglobin.

In certain additional preferred embodiments of the invention in this regard, the effector is determined by assaying an mRNA in cells of a tumor. In particularly preferred embodiments in this regard,

the mRNA encodes an inhibitor of PLA_2. Especially preferred is uteroglobin. In particularly preferred embodiments in this regard the tumor is a prostatic tumor and the inhibitor is uteroglobin.

In certain preferred embodiments in this regard, the mRNA is determined by a method comprising a step of hybridizing a probe to cells fixed on a surface. In certain preferred embodiments of this aspect of the invention the mRNA is determined by *in situ* hybridization.

In preferred embodiments of the invention in both regards the effector is an inhibitor of PLA_2, most particularly uteroglobin. In particularly preferred embodiments in this regard the tumor is a prostatic tumor and the inhibitor is uteroglobin.

In another aspect of the invention in this regard, aberrant mRNA is determined. In preferred embodiments of the invention in this regard, the mRNA encodes an inhibitor of PLA_2, most particularly uteroglobin. In particularly preferred embodiments in this regard the tumor is a prostatic tumor and the inhibitor is uteroglobin.

In a still further object of the invention there has been provided a kit for determining metastatic potential of a tumor. In certain preferred embodiments kits of the invention comprise: (A) a first reagent that binds specifically to an effector of arachidonic acid release in cells in a biopsy

sample prepared for determination of the effector, and (B) a second reagent for detectably labelling the primary binding reagent bound specifically to cells in the biopsy sample, wherein the determination of the effector tumor is diagnostic of the metastatic potential of the tumor.

In certain preferred embodiments of the kits of the invention, the effector is an inhibitor of PLA_2, among which uteroglobin is particularly preferred.

In certain further preferred embodiments of the this aspect of the invention, the first reagent is an antibody. In particularly preferred embodiments in this respect, the effector is an inhibitor of PLA_2, among which uteroglobin is particularly preferred.

Additional preferred embodiments of this aspect of the invention are those in which the first reagent is a hybridization probe. In certain preferred embodiments of this aspect of the invention, the effector is an inhibitor of PLA_2, among which uteroglobin is particularly preferred. Otherobjects, features and advantages of the invention will be apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while providing general and specific descriptions and indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the

invention will become apparent to those skilled in the art from the detailed description and other aspects of the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a bar chart showing the dose effect of uteroglobin on the invasiveness of cells of the TSU-Pr1 and DU-145 cell lines, epithelial cell lines derived from human prostate tumors. Both TSU-Pr1 and DU-145 cells are androgen independent. Cells were cultured in zero, 0.01, 0.1 and 1.0 μM uteroglobin for 24 hr. Invasiveness then was assayed by migration through filters coated with reconstituted basement membrane (RBM) in response to serum free or fibroblast conditioned media (FCM). Invading cells were stained with crystal violet, the dye was extracted and invasiveness was determined by measuring dye concentration by optical absorbance at 585 nm. Each point in the graph is the mean of results of three separate experiments, each carried out using triplicate cultures. The bars show the standard error of the mean for each point.

FIGURE 2 is a bar chart showing the dose effect of uteroglobin on the invasiveness of cells of the PC3-M and LNCaP cell lines, epithelial cell lines derived from human prostate tumors. LNCaP cells are

androgen-sensitive. PC3-M cells are androgen independent. Assays were performed as described in the caption to Figure 1.

FIGURE 3 is a bar chart showing that myoglobin, albumin and heat-inactivated uteroglobin do not affect the invasiveness of DU-145 cells. Assays were performed essentially as described in the caption to Figure 1.

FIGURE 4 is a graph showing a time course of the effect of uteroglobin on invasiveness of FCM-stimulated DU-145 cells. Cells were incubated without or with 1.0 μM uteroglobin for 3, 6, 12, or 24 hr. and then assayed for invasion in response to FCM as described in the caption to Figure 1.

FIGURE 5 is a graph showing that uteroglobin inhibits arachidonic acid release from FCM-stimulated DU-145 cells over a five hour period. Cells were incubated for 24 hr. in αMEM/SF media containing ^{14}C-labelled arachidonic acid. Free label then was washed away, and the cells were incubated in FCM without and with 1 μM uteroglobin. Arachidonic acid release was measured by ^{14}C released from the cells into the medium.

GLOSSARY

The abbreviations and terms in the present disclosure are employed in contemplation of their fullest meaning consistent with the disclosed and claimed invention. The following brief explanations are entirely illustrative and neither exhaustively define nor limit the invention disclosed and claimed herein. The full meaning of the terms will be clear from an understanding of the invention based on contemplation of the disclosure as a whole in light of a full understanding of the pertinent arts.

ARACHIDONIC ACID CASCADE: a series of enzymatic reactions that results in the production and release of arachidonic acid by a cell. The cascade is sensitive to ligand signals and arachidonic acid itself is an autocrine factor.

DU-145: an epithelial cell line derived from a human prostate tumor, which is androgen independent.

LNCaP: an epithelial cell line derived from a human prostate tumor, which is androgen sensitive. The LNCaP cell line was derived from a supraclavicular lymph node metastasis of a human prostate carcinoma. Cells of this line exhibit increased proliferation in response to androgen, *in vitro*, and they secrete prostate specific antigen (PSA), a marker of differentiated epithelial cells.

TSU-Pr1: an epithelial cell line derived from a human prostate tumor, which is androgen independent.

PC3-M: an epithelial cell line derived from a human prostate tumor, which is androgen independent.

EFFECTOR: a substance that engenders, alters, modulates or controls an activity of a cell; a substance that can engender, alter, modulate or control a physiological activity of a cell or organism. Typically, a protein, such as an enzyme, cofactor or transcription regulatory protein, or an activator or inhibitor of an enzyme, an enzyme complex, a receptor or a receptor complex, for instance.

EPITHELIAL CELL ORIGIN: derived from an epithelial cell, of whatever tissue.

FCM: fibroblast conditioned media

FIDUCIARY: a reference against which a test outcome is compared to gauge results. A fiduciary series is a plurality of such references that represent points along a qualitative or a quantitative scale.

IDENTIFYING: in the context of identifying PIN, ascertaining, establishing or otherwise determining one or more factual characteristics of prostatic intraepithelial neoplasia or a similar intermediate condition.

INHIBITION: inhibition of metastasis may be measured by many parameters in accordance with the

present invention and, for instance, may be assessed by delayed appearance of secondary tumors, slowed development of primary or secondary tumors, decreased occurrence of secondary tumors, slowed or decreased severity of secondary effects of disease, arrested tumor growth and regression of tumors, among others. In the extreme, complete inhibition, is referred to herein as prevention.

METASTASIS: As set out in Hill, R.P., Chapter 11, *Metastasis*, pp178-195 in *The Basic Science of Oncology*, Tannock *et al.*, Eds., McGraw-Hill, New York (1992), which is incorporated by reference herein in its entirety, metastasis is "The ability of cells of a cancer to disseminate and form new foci of growth at noncontiguous sites (*i.e.*, to form metastases)."

Similarly, metastasis is described in Aznavoorian *et al.*, *Cancer* 71: 1368-1383 (1993), which is incorporated by reference herein in its entirety, as "The transition from *in situ* tumor growth to metastatic disease is defined by the ability of tumor cells of the primary site to invade local tissues and to cross tissue barriers. ... To initiate the metastatic process, carcinoma cells must first penetrate the epithelial basement membrane and then invade the interstitial stroma. ... For distant metastases, intravasation requires tumor cell invasion of the subendothelial basement membrane that must also be negotiated during tumor cell extravasation ...

The development of malignancy is also associated with tumor-induced angiogenesis [which] not only allows for expansion of the primary tumor, but also permits easy access to the vascular compartment due to defects in the basement membranes of newly formed vessels."

MIMETIC: a molecule which, in shape and effect, mimics the shape and therefore the activity of another molecule or complex of molecules upon which it is designed.

MUTEIN: An amino acid sequence variant of a protein. The variation in primary structure may include deletions, additions and substitutions. The substitutions may be conservative or non-conservative. The differences between the natural protein and the mutein generally conserve desired properties, mitigate or eliminate undesired properties and add desired or new properties. In the present invention the muteins generally are those that maintain or increase anti-metastatic activity. Particularly, uteroglobin muteins are amino acid sequence variants of uteroglobin that maintain or increase the anti-metastatic activity of uteroglobin.

NSAID: Nonsteroidal anti-inflammatory agents. Small molecule drugs, as the term is used herein, that inhibit cyclooxygenase, but do not directly inhibit phospholipase A_2. These compounds have been used for their anti-inflammatory action. Aspirin,

phenylbutazone, ibuprofen, sulfinpyrazone (Anturane) and indomethacin are NSAIDs.

PEPTIDE ANALOG: an oligo or polypeptide having an amino acid sequence of or related to a protein. Peptide analogs of the present invention are peptides that have anti-metastatic activity and an amino acid sequence the same as or similar to a region of an anti-metastatic protein, such as uteroglobin.

PIN: prostatic intraepithelial neoplasia. A condition associated with prostate cancer having two grades, high grade PIN which may indicate a high risk of prostate cancer, and low grade PIN which may indicate a low risk of prostate cancer. PIN is also known as dysplasia, intraductal dysplasia, large acinar atypical hyperplasia, atypical primary hyperplasia, hyperplasia with malignant change, marked atypia, and duct-acinar dysplasia. Pin may be characterized by a high nuclear/cytoplasmic ratio, hyperchromasia, coarsely granular chromatin, absence of nucleoli, isolated cells and cellular and nuclear pleomorphism.

PLA: phospholipase A

PLA_2: phospholipase A_2

PREVENTION: in relation to metastasis, virtually complete inhibition, no metastasis if it had not occurred, no further metastasis if there had already been metastasis of a cancer. See INHIBITION.

PSA: Prostate specific antigen

RBM: reconstituted basement membrane. A multicomponent matrix approximating the molecular composition of the intracellular tissue matrix and the epithelial cell basement membrane. Preparations for preparing RBM are well known and are available from commercial suppliers.

SFM: Serum free medium

UG: uteroglobin

hUG: human uteroglobin

DETAILED DESCRIPTION OF THE INVENTION

Notwithstanding past failures to develop methods to identify prostatic intraepithelial neoplasia or to distinguish non-metastatic aberrations and tumors with low metastatic potential from aberrations and tumors with high metastatic potential, the present invention provides methods for determining the metastatic potential of aberrant growths, tumors and cancers and for identifying prostatic intraepithelial neoplasia.

In addition, notwithstanding past failures to develop effective anti-metastatic treatments, the present invention provides compounds and compositions for inhibiting cancer metastases and methods for administering the compounds and compositions to inhibit or prevent metastasis of a tumor of epithelial cell origin in an organism.

DETERMINING THE METASTATIC POTENTIAL OF TUMORS

Determining the activity of factors that effect arachidonic acid release in cells can serve to indicate metastatic potential of a tumor. In this regard, determining PLA_2 activity, and the activity or abundance of factors that affect the activity of PLA_2 can serve to indicate metastatic potential. Determining metastatic potential in accordance with this aspect of the present invention is illustrated by the following discussion of uteroglobin protein, mRNA or DNA as an index of metastatic potential of prostatic tumors, a very particularly preferred embodiment of the invention, which should not be construed as being limitative.

METHODS

Uteroglobin to index metastatic potential

The prostate is the sex gland in males that makes seminal fluid. It is located, in the standing male, vertically below the bladder, where it surrounds the urethra. Generally, it is shaped roughly like a slightly elongated sphere, like a walnut. In most men, it is about an inch in diameter until about age 50 and thereafter it tends to grow larger.

Pathology of the prostate can present as infection, benign enlargement or cancer. Benign

growth adversely affects health only when the enlarged prostate constricts the urethra and interferes with urination. Malignant growth always poses a threat to health and life of the patient; although, as discussed below, prognoses and indicated treatments vary greatly between occurrences.

Prostate cancer is the most frequently diagnosed cancer in men in the United States. Prostate cancers generally do not grow quickly. Usually, they double in size only every three or four years. Adverse affects of prostate cancer also develop slowly, as they are effected by the growth of the tumor itself.

The slow progression of prostate cancer presents something of a conundrum in men 50 or older, who present the majority of prostate cancer cases. Often the normal progression of prostate cancer suggests that debilitating effects will not develop within the normal life expectancy of the patient. Given the lack of effective treatments and the deleterious side effects attendant to the treatments currently available, waiting may be the best therapy for many elderly patients.

Unfortunately, it is difficult to distinguish benign from cancerous tumors and, more importantly, slow growing localized tumors from those formed by more aggressive, metastatic cancers. Thus, even the best physician cannot accurately predict the course of

progression of a given prostate cancer, and cannot prescribe the best treatment regimen.

In one aspect the present invention overcomes this obstacle to effective treatment of such tumors by providing a method to determine the metastatic potential of prostatic tumors. In accordance with this aspect of the invention, uteroglobin protein, mRNA or DNA is determined in cells of biopsy material. The protein, mRNA or DNA determined in the cells, by comparison to uteroglobin determined in normal cells, indicates the metastatic potential of prostatic tumors, particularly those of epithelial cell origin.

It is worth noting in this respect that previous studies did not identify the relationship between metastatic potential of a tumor and decreased expression of uteroglobin (or any other inhibitors of arachidonic acid release). In previous studies, for instance, uteroglobin (called Clara cell 10kDa protein, abbreviated CC10) was used as a marker for certain types of cells, and cell-type specificity of its expression was studied. (As described in Linnoila *et al.*, *A.J.C.P.* 97(2): 235-243 (1992) and Peri *et al.*, *J. Clin. Invest.* 92: 2099-2109 (1993), which are incorporated by reference herein in their entirety). In addition, CC10 expression was reported to vary between patients and cell types. In particular, it was reported that CC10 expression was lower in lung cancer patients and in smokers without lung cancer

than it was in non-smokers, and decreased CC10 expression has been loosely associated with neoplasm. (Broers *et al.*, *Lab. Invest.* 66: 337-346 (1992) and Jensen *et al.*, *Int. J. Cancer* 58: 629-637 (1994), which are incorporated by reference herein in their entirety. However, no studies of CC10 expression have suggested that uteroglobin expression in cells of a tumor can be used to determine metastatic potential.

Specific detection of proteins

Proteins indicative of metastatic potential of tumors can be determined in cells in biopsy material by conventional methods well known to those of skill in the art. Such methods are described in many standard textbooks and laboratory manuals. For instance, the techniques for making and using antibody and other immunological reagents and for detecting particular proteins in samples using such reagents are described in CURRENT PROTOCOLS IN IMMUNOLOGY, Coligan *et al.*, Eds., John Wiley & Sons, New York (1995), which is incorporated by reference herein in parts pertinent to making and using reagents useful for determining specific proteins in samples. As another example, immunohistochemical methods for determining proteins in cells in tissues are described in Volume 2, Chapter 14 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel *et al.*, Eds., John Wiley & Sons, Inc. (1994), which is incorporated by reference herein in

part pertinent to carrying out such determinations. Finally, Linnoila *et al.*, *A.J.C.P.* 97(2): 235-243 (1992) and Peri *et al.*, *J. Clin. Invest.* 92: 2099-2109 (1993),

incorporated herein as referred to above, describe techniques that may used, in part, in this aspect of the present invention.

For instance, uteroglobin can be determined in sample in accordance with the invention by histochemical methods set out in Miyamoto *et al.*, *J. Urology* 149: 1015-1019 (1993), which is incorporated by reference herein in its entirety. As described therein, for instance, suitable biopsy material is obtained from a patient suspected of having benign prostatic hyperplasia or prostatic carcinoma and immediately placed into 0.01M phosphate buffered saline. Thereafter, the material is immediately processed. It is mounted on a brass plate using rat liver homogenate as an adhesive. The material then is frozen in liquid nitrogen-cooled isopentane. Sections suitable for assay of uteroglobin in cells of the material are sectioned in a cryostat. Sections are obtained across the biopsy material, avoiding parts of the biopsy material that are damaged or deleteriously altered by the removal process.

Sections are dried at room temperature, fixed and then washed. Paraformaldehyde is a particularly useful fixative in this regard, but many other

fixatives also can be used. The sections may be pretreated with hydrogen peroxide and a non-ionic detergent, such as Triton X-100. Also, sections may be incubated with a blocking solution to reduce non-specific binding. For instance, the sections may be incubated with goat blocking serum prior to incubation with a goat serum, goat antibody or goat antibody-derived reagent.

Uteroglobin then is visualized for determination in the samples using a uteroglobin-specific binding reagent, such as a monoclonal or a polyclonal anti-uteroglobin antibody. Binding of the uteroglobin-specific reagent to cells in the sections may be determined directly, if the reagent has been conjugated to a detectable label, or using a second or additional reagents, such as a secondary antibody-enzyme conjugate.

In preferred embodiments of the invention, the uteroglobin-specific reagent is an antiserum, a polyclonal antibody, a derivative of a polyclonal antibody, a monoclonal antibody, a derivative of a monoclonal antibody or an engineered antibody, such as a single chain antibody. Derivatives of monoclonal and polyclonal antibodies include conjugates and fragments. Antibodies conjugated to detectable labels are preferred in this regard. Among detectable labels are enzymes such as horseradish peroxidase. Among

fragments preferred in this regard are Fab fragments, $F(ab')_2$ fragments and F(ab') fragments.

Sections are incubated with uteroglobin-specific reagent under conditions effective for the uteroglobin-specific reagent to bind efficiently to uteroglobin in said cells, while binding to other cellular components is inefficient; *i.e.*, under conditions effective for the ratio of specific to non-specific binding to provide accurate determination of uteroglobin content in cells of the biopsy material.

At the same time, control sections may be incubated under the same conditions with a corresponding reagent that is not specific for uteroglobin, to estimate background binding. For polyclonal immune serum, for instance, control sections can be incubated with preimmune serum to monitor background, non-specific binding. After the incubation period, the specific reagent, and any reagent used in the controls, is removed, as by washing.

If the primary, uteroglobin-specific reagent is detectably labelled, then the label may be determined and, thereby the uteroglobin content of cells in the sample. In this case, controls preferably would be labeled and would be determined in like fashion. More often, and preferably, a secondary reagent is used to visualize binding of reagents on the sections, as described below.

After removing unbound specific and non-specific reagents, test and control sections are incubated with a secondary reagent that binds specifically to the primary, uteroglobin specific reagent and its counterpart in the controls. Preferably, the secondary reagent is a biotinylated anti-antibody.

The sections are incubated with the secondary reagent under conditions for the reagent to bind efficiently to the primary reagent (and its counterpart in the controls) in the cells, while binding to other cellular components is inefficient; *i.e.*, under conditions effective for the ratio of specific to non-specific binding to provide accurate determination of uteroglobin content in cells of the biopsy material.

Thereafter, the unbound fraction of the secondary reagent is removed from the sections. The secondary reagent, and its counterpart in the controls, then is determined. If the secondary reagent comprises a detectable label, incubation with a tertiary reagent generally will not be necessary. However, use of a tertiary reagent comprising a detectable label is more commonly employed for immunocytochemical analysis, generally. Therefore, for illustrative purposes, the three component assay is described here.

The sections then are incubated with a tertiary reagent comprising a detectable label that binds specifically to the secondary reagent. Incubation is

carried out under conditions effective for the tertiary reagent to bind efficiently to the secondary reagent bound to primary reagent in cells in the test sections or its counterpart in control sections, while binding to other cellular components is inefficient; *i.e.*, under conditions effective for the ratio of specific to non-specific binding to provide accurate determination of uteroglobin content in cells of the biopsy material. A preferred tertiary reagent comprising a detectable label is an avidinated enzyme for binding to biotinylated secondary reagent. A preferred enzyme in this regard is horseradish peroxidase.

Unbound tertiary reagent is removed, by washing the sections with buffer, for instance. The detectable label bound in cells in the biopsy material then may be determined. In preferred embodiments of the invention, sections are incubated under conditions effective for an enzyme in the tertiary reagent to catalyze a chromogenic reaction. Binding of the uteroglobin-specific reagent is determined by the color generated by the reaction.

Suitable reagents and conditions for carrying out the determination of uteroglobin in cells in biopsy samples are well known and readily available. A multiplicity of procedures and reagents can be effectively employed for this purpose. Such reagents and techniques routinely are employed by those of

skill in the arts of immunocytochemistry, histopathology and cytology.

Kits for performing such assays, in whole and in part, are widely available from numerous commercial suppliers. Incubation with secondary antibodies, and subsequent visualization of uteroglobin, can carried out according the given procedures prescribed by commercial suppliers.

In preferred embodiments the sections are stained with hematoxylin and eosin to confirm pathology and to facilitate comparison of uteroglobin in normal and diseased cells in the same section.

In particularly, preferred embodiments, the relative staining of diseased and normal cells in a sections is compared with staining in fiduciary cells. The fiduciary cells are reference standards which typify results obtained by a given procedure in normal cells, cells characteristic of benign tumors, and cells characteristic of malignant tumors. Within any category, moreover, fiduciary cells may provide a graded series of characteristic results. Uteroglobin in fiduciary cells may be determined at the same time uteroglobin is determined in cells of the biopsy sample, or at another time. In a particularly preferred embodiment of the invention, uteroglobin is determined in fiduciary cells which serve as a standard reference series for subsequent clinical assays.

In normal tissue immunocytochemical techniques, such as those described above, reveal very heavy staining of uteroglobin in the luminal surface of prostatic epithelial cells. Biopsy samples that evidence intermediary pathology thought to precede neoplasia, such as prostatic intraepithelial neoplasia ("PIN"), show a pattern of uteroglobin staining similar but weaker than that of normal cells. Biopsy material from malignant tumors shows significant decreases in staining of uteroglobin in cells. The decrease in staining of the luminal surface of epithelial cells in prostatic tumors is particularly dramatic. Whereas, in normal tissue the luminal surface of epithelial cells shows the highest staining for uteroglobin, uteroglobin staining either cannot be detected or is faint in the same cells in metastatic prostatic tissue.

It is this ability to differentiate between normal tissue, prostatic intraepithelial neoplasia, and cancer which provides for the identification of PIN. Due to the association of PIN with cancer, especially high-grade PIN which has been reported to have a 70% association with cancer, this provides an early diagnosis, prognosis and treatment of prostate cancer.

Furthermore, it has been reported that there is a close relationship between the age-related prevalence of PIN and the age-related prevalence of

prostate cancer with the occurrence of PIN mirroring the occurrence of prostate cancer but having a 20-30 year lag time. It is this relationship which provides a practitioner the diagnostic and prognostic ability of early detection and treatment.

The risk of developing invasive cancer is gauged by the decrease in uteroglobin in diseased cells in the biopsy sample relative to cells in normal tissue of the same type, as described above. In biopsy material containing both normal and diseased tissue the staining of cells in the normal and diseased tissue can be compared on the same section. In general, the cells in low grade relatively confined tumors express uteroglobin in amounts similar to normal cells. The cells in aggressive, invasive tumors express little or no uteroglobin and are poorly differentiated in their morphology.

Specific detection of mRNA and DNA

mRNA also can be determined in cells in biopsy samples to determine metastatic potential. mRNA can be determined by a variety of methods well known to those of skill in the art, which can be carried out using well known and readily available starting materials, including those widely available from commercial suppliers. Techniques useful in this regard are described in the foregoing references. Techniques that may be particularly pertinent in this

regard relating to uteroglobin are described in Broers *et al.*, *Lab. Invest.* 66: 337-346 (1992) and Jensen *et al.*, *Int. J. Cancer* 58: 629-637 (1994), incorporated herein as referred to above.

A given mRNA may determined in cells of biopsy tissue by *in situ* hybridization to a specific probe. Such probes may be cloned DNAs or fragments thereof, RNA, typically made by *in vitro* transcription, or oligonucleotide probes, usually made by solid phase synthesis. Methods for making and using probe suitable for specific hybridization *in situ* are ubiquitously known and used in the art.

By specific hybridization is meant that the probe forms a duplex with the given, target mRNA that is stable to the conditions of hybridization and subsequent incubations and that duplexes formed between the probe and other, non-target mRNAs are not stable and generally do not persist through subsequent incubations. Specific hybridization thus means that the ratio of hybridization to target and non-target mRNAs provides an accurate determination of the target mRNA in cells in the biopsy sample.

In a particularly preferred embodiment of the present invention a probe that hybridizes specifically to uteroglobin mRNA is used to determine uteroglobin mRNA in cells of biopsy tissue, particularly prostatic biopsy material.

Techniques suitable for *in situ* determination of target mRNAs, such as uteroglobin mRNA, are described in a variety of well known and readily available laboratory manuals, as well as the primary literature. An illustrative procedure from the primary literature in this regard is described in Broers *et al.* *Laboratory Investigation* 66 (3): 337-346 (1992), which is incorporated by reference herein in its entirety.

In general, biopsy material is obtained by suitable surgical procedure and snap frozen, as by freezing in methybutane/dry ice. The samples can be embedded and sectioned much as described above for the determination of protein is biopsy samples. Sections can be thawed onto and affixed to glass slides previously cleaned with acid and ethanol and coated with poly-L-lysine. The tissue sections thereafter can be exposed to buffered formaldehyde, acetylated, treated with buffered glycine and then prehybridized in 50% formamide, 2X SSC (where 1X SSC is 0.15M NaCl, 0.015M sodium citrate, pH 7.0). After prehybridization the sections can be hybridized to the labelled probe in 50% formamide, 10% dextran sulfate, 2X SSC.

The exact conditions of the steps in the procedure, especially the prehybridization, hybridization and criterion steps will be adjusted with the T_m (or the T_d) of the probe and to provide the

desired degree of specificity of hybridization; *i.e.*, the desired stringency.

Theoretical approximations and empirical methods for determining proper conditions in this regard are well known and routinely practiced by those skilled in the pertinent arts. Approximation calculations and experimental techniques in this regard are described, for instance, in Sambrook *et al.* (1989) referred to herein above.

Those of skill will appreciate, for instance, that the formamide in the foregoing solutions serves to provide equivalent hybridization conditions at lower temperature. For instance, hybridization in 50% formamide at about 50°C provides conditions similar to hybridization at 65°C without formamide. The lower temperature of hybridization can help preserve the biopsy sections during the hybridization procedure, aiding subsequent identification and examination of cells and mRNA content. Other agents that preserve features of the tissue sections that aid analysis likewise are preferred.

Dextran sulfate generally is used to accelerate the hybridization reaction and to drive it to completion in a shorter period of time, as is well known. Similar agents that increase the rate of hybridization, consistent with accurate determination of specific mRNA content, also are useful in the present invention.

Following hybridization, the probe-containing solution and unbound probe are removed. Typically, the sections are washed several times with prehybridization buffer, such as 50% formamide, 2X SSC, at or slightly above the hybridization temperature.

If an RNA probe is used for detection of the target mRNA, the sections then are treated with RNAseA, typically in the same solution, and then washed to remove RNAseA and byproducts with 50% formamide, 2X SSC under the same conditions as the previous washings.

Finally, the sections typically are washed several additional times in 2X SSC at room temperature and then air dried.

Radioactive probes generally are visualized by autoradiography. For this purpose slides can be dipped in a photographic emulsion, dried and allowed to expose the emulsion at 4°C for an appropriate period of time. Using a preferred emulsion, NTB-2 nuclear track emulsion, exposure times of 3 to 7 days are appropriate. The exposure time can be altered by a variety of factors including the use of more highly labelled probes.

The emulsions are developed at the end of the exposure period and then, typically, counterstained with hematoxylin and eosin. Subsequently, labelling

of target mRNA in cells can be assessed by microscopy using brightfield and darkfield illumination.

As discussed above regarding protein, the abundance and distribution of the target mRNA in cells in the biopsy section indicates the metastatic potential of the tumor. Particularly, the relative abundance of mRNA in diseased and normal cells indicates metastatic potential.

A variety of controls may be usefully employed to improve accuracy in assays of this type. For instance, sections may be hybridized to an irrelevant probe and sections may be treated with RNAseA prior to hybridization, to assess spurious hybridization.

Thus, for instance, as discussed for uteroglobin protein, normal tissue exhibits high concentrations of uteroglobin mRNA in the prostatic epithelial cells. Intermediary pathology, such as prostatic intraepithelial neoplasia ("PIN"), shows a lower concentration of uteroglobin mRNA similar to but less than the hybridization to a uteroglobin mRNA-specific probe exhibited by normal cells. Biopsy material from malignant tumors shows significant decreases in the concentration of mRNA uteroglobin in cells by exhibiting little or no hybridization to a uteroglobin mRNA-specific hybridization probe.

Again, the differentiation between normal tissue, prostatic intraepithelial neoplasia, and cancer provides for the identification of PIN and an early

diagnosis, prognosis and treatment of prostate cancer.

aberrant mRNA or DNA

Some metastatic tissues of prostatic origin exhibit seemingly normal hybridization to a uteroglobin-specific probe, even though cells in the same tissue do not synthesize much, if any, uteroglobin protein. These cells typically exhibit aberrant uteroglobin mRNA, rather decreased uteroglobin mRNA. For instance, aberrant splicing has been demonstrated in at least one human prostatic carcinoma.

Splicing and other aberrations in mRNAs of cells of metastatic tissue can be determined by northern and southern blotting techniques and by PCR techniques. These techniques also are well known to those of skill in the art and can be applied readily to the determination of mRNA in cells of biopsy material in accordance with the present invention.

Techniques employed to assess restriction fragment length polymorphisms ("RFLP") can be applied to detect some mutations associated with aberrant splicing patterns. The assessment always can be made on the mRNA, but in some dysfunctions it can be made on the genomic DNA as well. The mRNA or DNA can be amplified prior to RFLP analysis, as well, using PCR or other suitable technique.

In addition to RFLP techniques, SSCP can be used to detect aberrant splicing of messages, such as uteroglobin-specific mRNA. For this purpose, a target mRNA, such as uteroglobin mRNA, is amplified by reverse transcriptase-mediated PCR. The double-stranded amplified DNA is denatured and run on gels in which mobility is quite sensitive to small changes in secondary structure.

Yet another technique that can be employed to determine aberrant splicing, among other things, is ligase mediated PCR. This technique also is well known to those of skill in the art, and techniques suitable to the analysis and determination of mRNAs and genomic aberrations that have been described in the literature readily can be applied to the determination of aberrant mRNAs in cells of tumor.

In regard to all of the foregoing, the determination of mRNA and genomic DNA in epithelial cells in biopsy material is preferred. Particularly preferred in this regard is prostatic biopsy material.

Among probes and hybridization targets for determination of metastatic potential of tumors by determination of target mRNA and DNA are probes specific for uteroglobin mRNA or for aberrations of uteroglobin mRNA or uteroglobin-encoding DNA indicative of altered expression of uteroglobin and, therefore, of metastatic potential.

FIDUCIARIES

Fiduciaries may be developed in accordance with this aspect of the invention, to guide interpretation of results. In this regard, a protein or mRNA in accordance with the foregoing may be determined in biopsy material from representative tumors of a specific type, characteristic of a specific degree of metastatic potential.

Characterization in this regard may benefit from hindsight, following the actual course of tumor progression in patients as they undergo treatment and thereafter. Fiduciary results characterizing a graded series of metastatic potential also may obtained from cell culture studies, as described elsewhere herein, illuminated in the examples below.

The determination of a protein or mRNA, or other agent, as set out above, in a variety of tumors of known metastatic characteristic, and the correlation of the determinations with metastatic potential is another preferred embodiment of the present invention.

KITS

Reagents for carrying out the methods described above may be incorporated into kits for use in determining the metastatic potential of a tumor or identifying prostatic intraepithelial neoplasia. All of the techniques and reagents discussed herein with regard to the determination of metastatic potential

and identifying prostatic intraepithelial neoplasia, including reagents and methods set out in the examples below may be included in these kits. Preferred kit components generally are those that detect the agents discussed elsewhere herein, particularly as discussed in the foregoing sections pertaining to determination of a protein or mRNA diagnostic of metastatic potential or PIN.

The kits also may include one or more fiduciary results, such as reference slides of immunocytochemical results characteristic of a tumors with high and low metastatic potential, or reference slides of *in situ* hybridization results characteristic in the same regard. Preferably in this regard, are kits that include a fiduciary series for interpreting results. The fiduciary may be in the form of one or more photographs or may be depicted in other ways, including written descriptions. In addition, the fiduciary may be highly tumor-type-specific or it may be applicable to related types of tumors.

ANTI-METASTATIC AGENTS AND METHODS

The invention disclosed herein provides agents and methods for inhibiting or preventing metastasis. The following discussion illustrates the invention in this respect.

Anti-metastatic agents

In particular, in accordance with this aspect of the invention, which include compounds that inhibit arachidonic acid release by cells of a tumor of epithelial cell origin in an organism are administered by a route and in an amount effective to prevent or inhibit metastasis of the tumor.

Inhibitors of arachidonic acid release

Without being limited to any theory of the invention, applicants note that arachidonic acid is a substrate in the synthetic pathway of eicosanoids in cells. Various eicosanoids play a role in stimulating or inhibiting shape, attachment, motility and proliferation of cells. In some aspects of the invention, inhibiting arachidonic acid release in cells of tumors of epithelial cell origin inhibits or extinguishes metastatic potential.

Inhibitors of phospholipase A_2

Compounds that inhibit phospholipase A_2 (PLA_2) are preferred compounds of the present invention. PLA_2 is a membrane signaling enzyme of the arachidonic acid cascade, the series of enzymes, substrates, products and co-factors involved in the production and secretion of arachidonic acid, and it generally will be the case that inhibitors of PLA_2 activity generally will inhibit release of arachidonic acid. Notably,

PLA_2 has been associated with processes of inflammation, rather than tumorigenesis or metastasis, and it has been suggested as a target for the control of chronic inflammation, but not as a target for developing an anti-metastatic agent. Nevertheless, the present invention provides compositions and methods of PLA_2 inhibitors for inhibiting metastasis of tumors of epithelial cell origin. The formulation and use of these compounds in the invention is illustrated by reference to the preferred embodiments discussed below.

Especially preferred among PLA_2 inhibitors are lipocortins, muteins of lipocortins, peptide analogs of lipocortins and uteroglobins, muteins of uteroglobins and peptide analogs of uteroglobin. Uteroglobins, muteins of uteroglobins and peptide analogs of uteroglobin are particularly preferred. Most particularly preferred are uteroglobins, and among these human uteroglobin is very especially preferred. The discussion below, directed to uteroglobin, particularly human uteroglobin, illustrates the invention is this regard.

Uteroglobins

Uteroglobin, also called blastokinin, was first discovered as a major protein component of the rabbit uterine fluid during early pregnancy. The human counterpart to rabbit uteroglobin was first found in

nonciliated Clara cells in the distal bronchiole airway and was originally designated Clara cell 10kD protein, abbreviated as "CC10." Uteroglobin also has been detected in humans in the uterus, respiratory tract, and prostate gland, by immunohistochemical methods.

The complementary DNA for human uteroglobin (CC10) has been cloned and its sequence has been determined, as reported in Singh *et al.*, *BBA* 950: 329-337 (1988), incorporated by reference herein in its entirety.

Uteroglobin has been purified to homogeneity by at least two groups and it has been structurally and functionally characterized in considerable detail. In brief, uteroglobin occurs in rabbits as a dimer of two identical chains. The monomers are 70 amino acids long. They are arranged antiparallel to one another in the dimer. Also, in the dimer they are covalently linked by two symmetrical disulfide bonds, formed between 'Cys-3' and "Cys-69" and, reciprocally, between 'Cys-69' and "Cys-3" (where ' designates one chain in the dimer and " designates the other chain). Each monomer chain contains four α-helical segments and a β-turn, the later at Lys-26 to Gln-29. The structure, function and activities of uteroglobin has been reviewed, for instance, by Miele *et al.*, *Endocrine Reviews* 8: 474-490 (1987), which is incorporated by reference herein in its entirety.

Uteroglobin inhibits the activity of PLA_2, as shown by *in vitro* assays. Generally, it has been thought to have immunomodulatory or anti-inflammatory activities, or both, that act to protect the wet epithelia of organs that communicate with the external environment. Uteroglobin expression is steroid-sensitive and its secretion in the endometrium has been shown to be stimulated by progesterone. Uteroglobin also has been reported to have an anti-chemotactic effect on neutrophils and monocytes. Uteroglobin has not been seen as playing a role in cancer or metastasis. Thus, it was surprising to find that uteroglobin, in accordance with the present invention, can be used to inhibit or prevent metastasis of a tumor of epithelial cell origin in an organism.

Without being bound to any theory of the mechanism by which uteroglobin inhibits metastasis, it appears, as the Examples show, that the inhibitory action of uteroglobin on metastasis results from inhibition of PLA_2 activity and inhibition of arachidonic acid release by the tumor cells. Any uteroglobin may be useful in the invention that inhibits arachidonic acid release that inhibits or prevents metastasis of a tumor of epithelial cell origin. Uteroglobins for use in the invention may be recovered from natural sources, it may be made by recombinant means, it may be produced by chemical

techniques, it may be made by semi-synthetic methods or it may be obtained by a combination of techniques.

Methods for purifying uteroglobin to homogeneity from a natural source have been described in Nieto *et al.*, *Arch. Biochem. Biophys.* 180:80-92 (1977), which is herein incorporated by reference in its entirety. Other methods for this purpose can be equally useful in this regard.

The most highly preferred uteroglobin for use in the invention at the present time is human uteroglobin. Preferably, human uteroglobin for use in the invention is made by expression of a cloned gene in a host cell in culture or in an animal. Techniques for expressing uteroglobin in this way are well known to those of skill in the art.

A cDNA encoding human uteroglobin, useful toward this end, has been isolated, sequenced and expressed in cells in culture. Methods for expressing cloned DNAs that encode uteroglobin have been described specifically with regard to human uteroglobin in Mantile *et al.*, *J. Biol Chem.* 268: 20343-20351 (1993) and Miele *et al.*, *J. Biol. Chem.* 265: 6427-6435 (1990), which, as noted below, are incorporated by reference herein in their entirety.

Techniques for obtaining, manipulating and expressing cloned genes to obtain uteroglobin for use in the present invention are well known to those of skill in the art and are described in protocol-like

detail in a variety of laboratory manuals. For instance, such methods are set forth in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2*ND* Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), the entirety of which is herein incorporated by reference.

Lipocortins

Lipocortin proteins also are known as annexins, and are well known to the art. They generally have been characterized as calcium-dependent phospholipid-binding proteins. For instance, Arcone *et al.*, *Eur. J. Biochem*, 211: 347-355 (1993), incorporated by reference herein in its entirety, reports on the structure of human lipocortin 1 and the expression of the active protein using an expression vector in *E. coli*.

Lipocortins have been implicated in the mechanism of anti-inflammatory activity of glucocorticosteroids. Furthermore, anti-inflammatory activity has been associated with the amino-terminal end of human lipocortin 1, as shown by the activity of acylated polypeptide corresponding to human lipoprotein residues 2-26, synthesized and characterized by Cirino *et al.*, *J. Pharmacol.* 108: 573-4 (1993), which is incorporated by reference herein in its entirety.

According to one hypothesis, lipocortins mediate a glucocorticosteroid-dependent inhibition of

phospholipase A_2. Some studies support a "substrate-depletion" mechanism of phospholipase A_2 inhibition, as reported by Bastian *et al.*, *J. Invest. Dermatol.* 101: 359-63 (1993), for instance, which is incorporated by reference herein in its entirety.

The studies have focused on the intermediary role of lipocortins in the anti-inflammatory response to glucocorticosteroids, however, and a role for the lipocortin proteins in metastasis was unknown.

In accordance with one aspect of the present invention, lipocortins may be used to inhibit or present metastasis of tumors of epithelial cell origin. The methods of using lipocortins, and the compositions of lipocortins, in accordance with the present invention, will be understood by reference to the discussion elsewhere herein, particularly by reference to the illustrative disclosure relating to uteroglobin and to prostate cancers.

Lipocortins generally may be used in accordance with this aspect of the present invention. Preferred lipocortins have a high therapeutic effect and low incidence of deleterious side effects. Particularly preferred lipocortins are those of human origin. Human lipocortin 1 is among those particularly preferred.

Muteins and peptide analogs

Techniques such as those described in the foregoing manual can be used to make variants and analogs of uteroglobin and other proteins useful in the invention. Recombinant DNA methods, chemical synthetic methods, enzymatic methods and mixed methods for making, altering and utilizing muteins and peptide analogs are well known and are described here only briefly to illustrate their applicability to the present invention.

-muteins

It will be appreciated by those of skill that cloned genes readily can be manipulated to alter the amino acid sequence of a protein. The cloned gene for human uteroglobin can be manipulated by a variety of well known techniques for *in vitro* mutagenesis, among others, to produce variants of the naturally occurring human protein, herein referred to as muteins, that may be used in accordance with the invention.

The variation in primary structure of muteins of lipocortins or uteroglobins useful in the invention, for instance, may include deletions, additions and substitutions. The substitutions may be conservative or non-conservative. The differences between the natural protein and the mutein generally conserve desired properties, mitigate or eliminate undesired properties and add desired or new properties. In the

present invention the muteins generally are those that maintain or increase anti-metastatic activity. Particularly, uteroglobin muteins are amino acid sequence variants of uteroglobin that maintain or increase the anti-metastatic activity of uteroglobin.

-peptide analogs

Similarly, techniques for making small oligopeptides and polypeptides that exhibit activity of larger proteins from which they are derived (in primary sequence) are well known and have become routine in the art. Thus, peptide analogs of proteins of the invention, such as peptide analogs of lipocortin and uteroglobin that exhibit anti-metastatic activity also are useful in the invention.

Mimetics

Mimetics also can be used in accordance with the present invention to prevent or inhibit metastasis of tumors. The design of mimetics is known to those skilled in the art, and generally are understood to be peptides or other relatively small molecules that have an activity the same or similar to that of a larger molecule, often a protein, on which they are modeled.

Thus, by way of illustration, uteroglobin mimetics, for instance, can be used in accordance with the present invention in the same manner as

uteroglobin itself, to prevent or inhibit metastasis of a tumor.

The design of such mimetics can be based on the structure-function relationship of uteroglobin. By studying the effect of mutations on anti-metastatic activity of uteroglobin the sites in the protein responsible for anti-metastatic activity can be identified. *In vitro* mutagenesis procedures that can be used to systematically alter cloned genes, such as cDNAs encoding uteroglobin and other proteins with anti-metastatic activity of the present invention, are described in Sambrook *et al.* (1988). Systematically mutagenized proteins, also referred to as muteins as noted elsewhere herein, can be produced using such altered DNA by standard methods for expression cloned genes in organisms to produce heterologous proteins. Such methods are well known to those of skill and are described in, for instance, Sambrook *et al.* (1988) referred to herein above. The muteins so produced then can be assayed for anti-metastatic activity, using *in vitro* or *in vivo* assays that model or measure metastatic activity. Suitable methods are described herein and illustrated in the examples below.

Methods for determining aspects of protein structure also are well known to those of skill in the art. To some extent, the structure of a given protein can be approximated by analogy to structures of related proteins. Physical and chemical information

about a protein structure can be obtained by a wide variety of well known techniques, including active site modification techniques, NMR, and X-ray crystallography.

This information can be combined with information from studies that correlate structural alterations with changes in activity, such as the mutagenesis studies described above, to generate a map of the shape and chemical functions important to a given activity of a protein.

Molecules that mimic the shape and chemical functionality that provide the desired activity, then can be designed and synthesized. Computer modeling methods that can be employed toward this end, as well as methods of organic synthesis, peptide synthesis and for the synthesis of other classes of compounds that can be used to produce mimetics in accordance with this aspect of the invention are well known to those of skill in the art.

Once a mimetic has been designed and synthesized, it can be assayed for anti-metastatic activity using techniques for this purpose, such as those described elsewhere herein.

Results of activity studies and of structural studies of the mimetics themselves can be used to design further mimetics that are more effective, have fewer undesirable side effects, or have additional

activities, such as by combining two mimetics in a single molecule.

In the same manner as for uteroglobin, mimetics can be designed for other compounds that have anti-metastatic activity. Preferred in this regard, as described above, as anti-metastatic mimetic compounds that inhibit arachidonic acid release by cells of a cancer of epithelial cell origin. Particularly preferred are mimetics that inhibit phospholipase A_2 in such cells. In this regard, mimetics of uteroglobin or lipocortin are especially preferred. Among the most highly preferred mimetics in this regard are mimetics of human uteroglobin.

Small molecule drugs

Compounds other than the proteins, muteins, protein-derived peptides, mimetics and the like discussed above, that inhibit arachidonic acid release by cells of cancers of epithelial cell origin also may be useful in the present invention. Among such compounds are certain small organic molecules, which may be mimetics, that inhibit arachidonic acid release. Inhibition may be mediated by inhibition of PLA_2 activity, or by inhibition of other enzymes or intermediates involved in metabolic interactions that result in arachidonic acid release.

Among such compounds is the anti-inflammatory agent mepacrine, which has been shown to inhibit PLA_2,

and an experimental drug, indomethacin, which has been shown to inhibit cyclooxygenase. Both compounds and exhibit anti-metastatic activity in *in vitro* assays, at doses that have been shown to be non-toxic in patients. Both compounds, thus, can be utilized in accordance with the present invention.

PLA_2, as noted herein above, is a key enzyme in the arachidonic acid cascade. As noted above, inhibitors of PLA_2 are most preferred in the invention, in this regard. Among small molecule drugs, mepacrine is preferred among inhibitors of PLA_2. Other relatively small molecule drugs (small, in this case, meaning small relative to proteins of average size) that, like mepacrine, inhibit PLA_2 also will be useful in the invention, in the same fashion as mepacrine and the other PLA_2 inhibitors discussed herein above. Among small molecule drugs, such PLA_2 inhibitors are particularly preferred. In this regard, mepacrine is highly preferred and other compounds that are similar to mepacrine in chemical structure are particularly preferred.

Cyclooxygenase is the key enzyme in the cyclooxygenase-dependent pathway of arachidonic acid metabolism, wherein arachidonic acid is a precursor in the synthesis of prostaglandins, prostacyclins and thromboxanes. Among small molecule drugs, inhibitors of cyclooxygenase also are preferred for use in the invention disclosed herein. Nonsteroidal anti-

inflammatory agents ("NSAIDs") are among the small molecule drugs, as the term is used herein, that inhibit cyclooxygenase and are preferred in the invention in this regard. NSAIDs are described, for instance, in PRINCIPLES OF PHARMACOLOGY, Munson *et al.*, EDs., Chapman & Hall, New York (1995), which is incorporated herein by reference in part pertinent thereto, including, particularly, Chapter 74.

Among NSAIDs in accordance with this aspect of the invention are aspirin, phenylbutazone, ibuprofen, sulfinpyrazone (Anturane) and indomethacin. In this regard, indomethacin is particularly preferred and compounds that are similar to indomethacin in chemical structure also are preferred.

Lipoxygenase is the key enzyme in the lipoxygenase-dependent pathway of arachidonic acid metabolism, wherein arachidonic acid is a precursor in the synthesis of thromboxanes. Inhibitors of lipoxygenase, and other downstream enzymes of the lipoxygenase-dependent pathway also may be of use in the present invention.

Compositions

Any non-toxic, inert and effective carrier may be used to formulate compositions of the present invention. Well known carriers used to formulate other therapeutic compounds for administration to humans particularly will be useful in the compositions

of the present invention. Pharmaceutically acceptable carriers, excipients and diluents in this regard are well known to those of skill, such as those described in the MERCK INDEX, 11th Ed., Budavari *et al.*, Eds., Merck & Co., Inc., Rahway, New Jersey (1989), which is incorporated by reference herein in its entirety. Examples of such useful pharmaceutically acceptable excipients, carriers and diluents include distilled water, physiological saline, Ringer's solution, dextrose solution, Hank's solution and DMSO, which are among those preferred for use in the present invention.

In particular, for instance Mantile *et al.*, *J. Biol Chem.* 268: 20343-20351 (1993), incorporated by reference hereinabove, report on sterile formulated, lyophilized uteroglobin that may be useful in preparing uteroglobin compositions of the invention.

Cancers

Methods and compositions of the present invention may be applied to the treatment of a variety of cancers of epithelial cell origin. Among these are metastatic cancers of breast, lung, colon, bladder, prostate, gastrointestinal track, endometrium, tracheal-bronchial tract, pancreas, liver, uterus, nasopharynges and the skin. An especially preferred target is prostate cancer, particularly prostate cancer of epithelial cell origin.

The following detailed discussion of prostate cancers is provided in illustration of the compositions and methods of the invention not only as to prostate cancers, but also other cancers that may be treated in analogous or identical fashion, in accordance with the present invention.

Prostatic adenocarcinoma

Adenocarcinoma of the prostate is one of the most common malignancies. It is estimated that 240,000 new cases of prostate cancer will be diagnosed in the United States in 1995, and that it will cause more than 50,000 deaths during the year. In fact, prostate adenocarcinoma is the second leading cause of cancer-related mortality in the United States.

With prostate cancer, as with all solid tumors, it is the metastatic encroachment of the tumor on other vital function that causes the demise of the patient. Approximately 10% of patients are diagnosed initially with metastatic disease. Ultimately, 30-40% of patients with this cancer will develop metastatic disease. Once metastasis occurs there is a the cancer follows a relentless progression.

Invasion is a prerequisite for migration of tumor cells. In connective tissue, stroma and basement membranes form the major physical barriers to the migration process. Invasion of the local extracellular matrix (ECM) by tumor cells thus can be

marked as the first step in metastasis. The sequential biochemical mechanism of ECM invasion first involves cell attachment to specific components of ECM followed by a progressive cascade of proteolytic dissolution. Prostate cancers which grow to a critical size exhibit extracapsular invasion and metastasize to specific anatomical sites apparently in response to stromal cell secretory proteins which are necessary for their growth and proliferation. Invasive migration of tumor cells within the prostate gland may occur as a function of chemokinesis along anatomical paths of least resistance, such as the perineural duct. Further establishment of metastasis relies upon successful penetration of the circulatory or lymphatic system, followed by vessel extravasation at the secondary organ, which frequently is bone tissue for cancers of prostatic origin. Nearly all of these steps, including attachment, matrix degradation and migration, can be modeled experimentally *in vitro* by measuring invasion of a reconstituted basement membrane (RBM) barrier in response to fibroblast-conditioned medium (FCM) which serves as a chemo-attractant.

In vivo, of growth and proliferation of prostate tumor cells primarily is responsive to stromal cell (fibroblast) secretory proteins. Extracapsular invasiveness of prostate tumor cell can be modeled by migration of tumor cells *in vitro* into reconstituted

basement membrane (RBM) in the presence and absence of a chemoattractant, such as fibroblast conditioned medium (FCM). The assay determines cells that have attached to the RBM, degraded the RBM enzymatically and, finally, cells that have towards the FCM side of the membrane. The events in the *in vitro* invasion assay comport with the important steps observed in the metastatic spread of tumor cells through the basement membrane *in vivo*.

Prostate tumors frequently initially metastasize to regional lymph nodes, having disseminated through the lymphatic circulation. They also spread to other sites through the vascular system, which is extensively interconnected to the lymphatics. The final site of formation of metastasis is a function of a number of parameters, including: (i) the first capillary bed encountered by blood vessels draining the tumor, and (ii) organ preference of the tumor cells with respect to characteristics of specific tissues that nurture attachment and growth of tumor cells with metastatic potential.

Metastatic potential of prostate cancers of epithelial cells origin can be inhibited by compositions and methods of the invention. In particular, metastasis of these cancers can be inhibited by human uteroglobin, as shown by the examples set out herein below.

Route of administration

Therapeutic treatment with uteroglobin can utilize any type of administration including topical, other non-invasive and invasive means.

Administration by non-invasive means may be by oral, intranasal or transdermal routes, among others.

Generally, at the present time, invasive techniques are preferred. Administration by invasive techniques may be intravenous, intraperitoneal, intramuscular or directly in tumors, among others.

Administration may be by a single dose, it may be repeated at intervals or it may be continuous. Since uteroglobin is small, easily diffusible, and relatively stable it is well suited to long-term continuous administration, such as by perfusion pump. Where continuous administration is applied, infusion is preferred. In this situation, pump means often will be particularly preferred for administration. Especially, subcutaneous pump means often will be preferred in this regard.

In other situations it will be desirable to administered uteroglobin and other agents of the present invention by intramuscular self-injection on a regular basis.

Compositions and methods of the invention also may utilize controlled release technology. Thus, for example, uteroglobin may be incorporated into a hydrophobic polymer matrix for controlled release over

a period of days. Such controlled release films are well known to the art. Examples of polymers commonly employed for this purpose that may be used in the present invention include nondegradable ethylene-vinyl acetate copolymer and degradable lactic acid-glycolic acid copolymers. Certain hydrogels such as poly(hydroxyethylmethacrylate) or poly (vinylalcohol) also may be useful, but for shorter release cycles then the other polymer releases systems, such as those mentioned above.

Dose

The quantity of the active agent for effective therapy will depend upon a variety of factors, including the type of cancer, means of administration, physiological state of the patient, other mendicants administered, and other factors.

Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from *in vitro* studies initially will provide useful guidance on the proper doses for patient administration. Studies in animal models also generally may be used for guidance regarding effective dosages for treatment of metastatic cancers in accordance with the present invention.

These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard

textbooks, such as GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th Ed., Gilman *et al.* Eds. Pergamon Press (1990) and REMINGTON'S PHARMACEUTICAL SCIENCES, 17th Ed., Mack Publishing Co., Easton, Pa. (1990), both of which are incorporated by reference herein in their entirety.

Typical therapeutic doses will be about 0.1 to 1.0 mg/kg of body weight of pure uteroglobin. The does may be adjusted to attain, initially, a blood level of about 0.1 μM.

A particular formulation of the invention uses a lyophilized form of uteroglobin, in accordance with well known techniques. For instance, 1 to 100 mg of uteroglobin may be lyophilized in individual vials, together with carrier and buffer compound, for instance, such mannitol and sodium phosphate. The uteroglobin may be reconstituted in the vials with bacteriostatic water and then administered, as described elsewhere herein.

Administration regimen

Any effective treatment regimen can be utilized and repeated as necessary to affect treatment.

In clinical practice, the compositions containing uteroglobin or recombinant uteroglobin, alone or in combination with other therapeutic agents are administered in specific cycles until a response is obtained.

For patients who initially present without metastatic disease, uteroglobin-based drugs can be used as an immediate initial therapy prior to surgery and radiation therapy, and as a continuous post-treatment therapy in patients at risk for recurrence or metastasis (based upon high PSA, high Gleason's score, locally extensive disease, and/or pathological evidence of tumor invasion in the surgical specimen). Therapy for these patients aims, for instance, to decrease the escape of potentially metastatic cells from the primary tumor during surgery or radiotherapy, decrease the escape of tumor cells from undetectable residual primary tumor, decrease tumor cell attachment to the interior vessel wall, decrease the migration of tumor cells out of the vessel, and thereby decrease invasion into the interstitial spaces of the distal organ.

For patients who initially present with metastatic disease, uteroglobin-based drugs can be used as a continuous supplement to, or possible as a replacement for hormonal ablation. A goal of therapy for these patients is to slow tumor cell escape from both the untreated primary tumor and from the existing metastatic lesions in order to slow the progressive encroachment of further metastases.

In addition, the invention may be particularly efficacious during post-surgical recovery, where the present compositions and methods may be particularly

effective in lessening the chances of recurrence of a tumor engendered by shed cells that cannot be removed by surgical intervention.

Gene therapy

Certain embodiments of the present invention relate to anti-metastatic gene therapy. Gene therapy is a new approach to treatment of diseases. Currently, gene therapy protocols relate to therapy of certain carefully chosen disorders, including certain inherited disorders, a number of aggressively fatal cancers and AIDS. The restricted application of gene therapy to a few disorders reflects concerns about the efficacy, safety and ethical implications of the approach in general, and current techniques in particular. Despite the cautious approach mandated by these concerns, and despite the fact that techniques for carrying out gene therapy are still in an early stage of development, results from the first few trials have been very encouraging, some spectacularly so. It seems certain that gene therapy techniques will improve rapidly and that gene therapies soon will develop into an increasingly important and ubiquitous modality for treating disease. (Reviewed, for instance, in Tolstoshev, *Ann. Rev. Pharm. Toxicol.* 32: 573-596 (1993) and Morgan *et al.*, *Ann. Rev. Biochem.* 62: 191-217 (1993), which are incorporated by reference herein in their entirety).

The delivery of a variety of therapeutic agents clearly will be accomplished by gene therapy techniques. Many of the procedures now in use or under current development for gene therapy may be used in accordance with the present invention to prevent or inhibit metastasis. Additional techniques that will be developed in the future similarly will be found useful in the present invention. The following discussion is illustrative of the use of gene therapy techniques to prevent or inhibit metastasis in accordance with the present invention.

By gene therapy, in the following discussion, generally is meant the use of a polynucleotide, in a cell, to achieve the production of an agent and the delivery of the agent to a tumor *in situ*, *i.e.*, in a patient, to engender an anti-metastatic effect. The agent may itself be a anti-metastatic agent or it may engender the production of an anti-metastatic agent upon introduction into the patient.

Approaches to genetic therapy currently being developed, which can be used in accordance with this aspect of the invention disclosed herein, often are grouped into two major categories: *ex vivo* and *in vivo* techniques.

Ex vivo techniques generally proceed by removing cells from a patient or from a donor, introducing a polynucleotide into the cells, usually selecting and growing out, to the extent possible, cells that have

incorporated, and, most often, can express the polynucleotide, and then introducing the selected cells into the patient. Cells that target tumor cells *in vivo*, including tumor cells that have migrated from primary or secondary tumor sites, generally are preferred in this type of gene therapy.

In addition, as described further below, the polynucleotide may be introduced directly into the patient. The polynucleotide in this case may be introduced systemically or by injection into a tumor site. The polynucleotide may be in the form of DNA or RNA, alone or in a complex, or in a vector, as discussed further below.

The polynucleotide may be in any of a variety of well-known forms, for instance, a DNA, a DNA fragment cloned in a DNA vector, a DNA fragment cloned in DNA vector and encapsidated in a viral capsid.

The polynucleotide may be an RNA or a DNA. More typically it is a DNA. It may include a promoter, enhancer and other *cis*-acting control regions that provide a desired level and specificity of expression in the cells of a region operably linked thereto that encodes an RNA, such as an anti-sense RNA, or a protein. The polynucleotides may contain several such operably linked control and encoding regions for expression of one or more mRNAs or proteins, or a mixture of the two.

Preferred in this regard are polynucleotides that encode the anti-metastatic agents described herein above. As noted in the foregoing discussion, inhibitors of arachidonic acid release are preferred. Inhibitors of PLA_2 activity are particularly preferred. Among PLA_2 inhibitors, uteroglobins and lipocortins are particularly preferred, uteroglobins especially, human uteroglobin particularly among uteroglobins.

Muteins and polypeptide analogs of protein inhibitors also are useful in the invention and may be encoded by polynucleotides for gene therapy to inhibit metastasis. In this regard, muteins and polypeptide analogs of the foregoing preferred embodiments also are preferred in this aspect of the invention.

In addition, peptide mimetics that can be encoded by a polypeptide for synthesis in cells can be used in accordance with this aspect of the invention. Preferred embodiments in this regard those set out above.

The polynucleotide may be introduced into cells either *ex vivo* or *in vivo*, including into the tumor *in situ*. A variety of techniques have been designed to deliver polynucleotides into cells for constitutive or inducible expression, and these routine techniques can be used in gene therapy of the present invention as well. Polynucleotides will be delivered into cells *ex vivo* using cationic lipids, liposomes or viral vectors. Polynucleotides will be introduced into

cells *in vivo*, including into cells of tumors *in situ*, using direct or systemic injection. Methods for introducing polynucleotides in this manner can involve direct injection of a polynucleotide, which then generally will be in a composition with a cationic lipid or other compound or compounds that facilitate direct uptake of DNA by cells *in vivo*. Such compositions may also comprise ingredients that modulate physiological persistence. In addition, polynucleotides can be introduced into cells *in vivo* in viral vectors.

Genetic therapies in accordance with the present invention may involve a transient (temporary) presence of the gene therapy polynucleotide in the patient or the permanent introduction of a polynucleotide into the patient. In the latter regard, gene therapy may be used to repair a dysfunctional gene to prevent or inhibit metastasis.

Genetic therapies, like the direct administration of agents discussed above, in accordance with the present invention may be used alone or in conjunction with other therapeutic modalities.

Combined with other treatments

Uteroglobin may be used in conjunction with other treatment modalities. Other common treatment modalities are discussed below specifically by reference to prostate cancer. It will be appreciated

that similar consideration will apply to treatment of other metastatic cancers. The present invention may be used in conjunction with any current or future therapy.

Surgery and Radiation

In general, surgery and radiation therapy are employed as potentially curative therapies for patients under 70 years of age who present with clinically localized disease and are expected to live at least 10 years. Neither treatment modality has a significant role in the management of metastatic diseases, and neither treatment is generally performed if metastasis is present at initial diagnosis.

Approximately 70% of newly diagnosed prostate cancer patients fall into this category. Approximately 90% of these patients (63% of total patients) undergo surgery, while approximately 10% of these patients (7% of total patients) undergo radiation therapy.

Histopathological examination of surgical specimens reveals that approximately 63% of patients undergoing surgery (40% of total patients) have locally extensive tumors or regional (lymph node) metastasis that was undetected at initial diagnosis. These patients are at a significantly greater risk of recurrence or metastasis. Approximately 40% of these patients will actually develop recurrence or

metastasis within 5 years after surgery. Results after treatment with radiation are even less encouraging. Approximately 80% of patients who have undergone radiation as their primary therapy have disease persistence or develop recurrence or metastasis within 5 years after treatment.

Currently, surgical and radiotherapy patients generally do not receive any immediate follow-up therapy. Rather, they typically are monitored for elevated Prostate Specific Antigen ("PSA"), which is the primary indicator of recurrence or metastasis.

Thus, there is considerable opportunity to use the present invention in conjunction with surgical intervention.

Hormonal Therapy

Hormonal ablation is the most effective palliative treatment for the 10% of patients presenting with metastatic disease at initial diagnosis. Hormonal ablation by medication and/or orchiectomy is used to block hormones that support the further growth and metastasis of prostate cancer. With time, both the primary and metastatic tumors of virtually all of these patients become hormone-independent and resistant to therapy. Approximately 50% of patients presenting with metastatic disease die within 3 years after initial

diagnosis, and 75% of such patients die within 5 years after diagnosis.

In this regard, it may be worth noting that the natural uteroglobin gene is dependent upon hormones for expression, and hormonal ablation may decrease expression of the endogenous uteroglobin gene, both in tumor cells and in the normal tissue surrounding the tumor. A uteroglobin-deficient state could render the patient more susceptible to successful metastasis. Continuous supplementation with uteroglobin-based drugs may be used to prevent or reverse this potentially metastasis-permissive state from developing in hormonal therapy treatment modalities.

Chemotherapy

Chemotherapy has been more successful with some cancers than with others. It is likely that the combination of chemotherapy with therapies of the present invention in some cases will be synergistic. Chemotherapy currently has little effect on prostate cancer and is used only as a last resort, with universally dismal results.

Immunotherapy

The present invention also can be used in conjunction with immunotherapies. Not only may the methods and compositions herein disclosed be used with the increasing variety of immunological reagents now

being tested or used to treat cancer, but it also may be used with those that come into practice in the future. The present invention thus may be used with immunotherapies based on polyclonal or monoclonal antibody-derived reagents, for instance. Monoclonal antibody-based reagents are among those most highly preferred in this regard. Such reagents are well known and are described in, for instance, Ritter MONOCLONAL ANTIBODIES - PRODUCTION, ENGINEERING AND CLINICAL APPLICATIONS, Ritter *et al.*, Eds., Cambridge University Press, Cambridge, UK (1995), which is incorporated by reference herein in its entirety. Radiolabelled monoclonal antibodies for cancer therapy, in particular, also are well known and are described in, for instance, CANCER THERAPY WITH RADIOLABELLED ANTIBODIES, D.M. Goldenberg, Ed., CRC Press, Boca Raton, FL (1995), which is incorporated by reference herein in its entirety.

Cryotherapy

Cryotherapy recently has been applied to the treatment of some cancers. Methods and compositions of the present invention also can be used in conjunction with an effective therapy of this type.

Compositions comprising several active agents

According to another aspect of the invention, pharmaceutical compositions of matter useful for inhibiting cancer metastases are provided that

contain, in addition to the aforementioned compounds, an additional therapeutic agent. Such agents may be chemotherapeutic agents, ablation or other therapeutic hormones, antineoplastic agents, monoclonal antibodies useful against cancers and angiogenesis inhibitors. The following discussion highlights some agents in this respect, which are illustrative, not limitative. A wide variety of other effective agents also may be used.

Among hormones which may be used in combination with the present invention diethylstilbestrol (DES), leuprolide, flutamide, cyproterone acetate, ketoconazole and amino glutethimide are preferred.

Among antineoplastic and anticancer agents that may be used in combination with the invention 5-fluorouracil, vinblastine sulfate, estramustine phosphate, suramin and strontium-89 are preferred.

Among the monoclonal antibodies that may be used in combination with the invention CYT356 is preferred.

The present invention is further described by reference to the following, illustrative examples.

EXAMPLE 1 Preparation of human uteroglobin by gene expression

Human uteroglobin was purified from *E. coli* cells expressing a full-length cDNA. The methods for obtaining the cDNA, constructing it into a vector for expression in host, expressing the construct and purifying the protein all involve art routine techniques. Such methods are described specifically with regard to human uteroglobin in Singh *et al.*, *BBA* 950: 329-337 (1988), Mantile *et al.*, *J. Biol Chem.* 268: 20343-20351 (1993) and Miele *et al.*, *J. Biol. Chem.* 265: 6427-6435 (1990), which are incorporated by reference herein in their entirety.

Briefly, in the present illustrative example, which followed the techniques set out in the foregoing

references, a clone containing a full-length cDNA encoding human uteroglobin in the well known vector pGEM4Z was digested with *Pst*I. (pGEM4Z may be obtained from Promega, Inc. Many other equally suitable vectors also are available commercially.) The digestion freed a 340-base pair fragment containing all of the cDNA and 53 nucleotides of the pGEM4Z polylinker. The fragment was purified by preparative gel electrophoresis in low melting temperature agarose. The purified fragment was ligated into the *Pst*l site of the expression vector pLD101, downstream of an inducible promoter. The ligation and subsequent cloning produced the plasmid pGEL101. This construct was introduced into *E. coli* strain BL21(DE3) cells for expression of uteroglobin protein.

For expression, bacteria were cultured under routine conditions for *E. coli* growth, and then induced for uteroglobin expression by making the media 0.45 mM in IPTG (isopropyl-1-thio-D-galactopyranoside). After appropriate further incubation to accumulate expressed protein, the cells were collected and then lysed. Uteroglobin was purified from the lysed cells using standard methods of size exclusion and ion exchange chromatography.

EXAMPLE 2 Cells for assays of metastatic potential

The cell lines used in the illustrative embodiments herein discussed are well known and readily available. The four cell lines of the present examples all were derived from human prostate cancer and are of epithelial cell origin. TSU-Pr1, DU-145 and PC3-M are androgen-independent. LNCaP is androgen-sensitive.

DU-145 is described in Stone *et al.*, *Int. J. Cancer* 21: 274-281 (1978), which is incorporated herein by reference in its entirety. The cell line is

available from a variety of sources including, for instance, the American Type Culture Collection (Rockville, MD).

LNCaP is described in Horoszewicz *et al.*, *Cancer Res.* 43: 1809-1818 (1983), which is herein incorporated by reference in its entirety. This cell line may be obtained from, for instance, the American Type Culture Collection (Rockville, MD).

PC3-M is described in Kaighn *et al.*, *Invent. Urol.* 11:16-23 (1976) which is herein incorporated by reference in its entirety.

TSU-Pr1 is described in Hzumi *et al.*, *J. Urology* 137:1304-1306 (1987) which is incorporated by reference in its entirety.

Cells of each line were grown and maintained in monolayer culture in αMEM (minimal essential medium) supplemented with glutamine, 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 (g/ml). Cultures were incubated at 37°C in 5% CO_2/95% air. Media was replaced every second day.

EXAMPLE 3 General assay for invasiveness of cells

1. Culture

As described briefly below, invasiveness of cells was assayed by the methods described in Albini *et al.*, *Cancer Research* 47: 3239-3245 (1987), which is incorporated herein by reference in its entirety. Invasiveness assays and other methods for assessing anti-metastatic affects, as discussed herein below are described in Leyton *et al.*, *Cancer Research* 54:3696-3699 (1994) which is incorporated by reference herein in its entirety.

Cells in logarithmic phase were detached from the growth surface by brief exposure to 0.25% trypsin, 0.25% EDTA, collected and centrifuged at 800 X g for 5 min. The pellet was resuspended in SF medium,

counted and seeded into 6 mm dishes, 1.5 X 10^6 cells per dish. The cells then were incubated for 24 hr in media containing zero, 0.01, 0.1 and 1.0 μM uteroglobin. After the incubation cells were gently collected using a rubber policeman and assayed for invasiveness.

Fibroblast conditioned media (FCM) served as a chemo-attractant to stimulate invasion. It was prepared by culturing proliferating 3T3 cells for 24 hr. in SF medium and then collecting the media, free of cells. The cell free media thus obtained served as FCM.

Invasiveness was measured using a polycarbonate membrane precoated with a reconstituted basement membrane. Well known RBMs are suitable for this purpose. For example, Albini *et al.*, supra describes RBM of the type employed for these experiments.

Assays were performed in blind-well Boyden chambers. The lower compartment of each chamber was filled with 220 μl of FCM, as chemo-attractant, or 220 μl serum-free media, as control for basal invasiveness. A polycarbonate membrane (12-μm pore size), coated with 25 μg/50 μl RBM, was placed over the lower compartment. Tumor cells for assay were added to the upper compartment, 3.0 X 10^5 cells per well, and the chambers then were incubated at 37°C for 6 hr.

(Reconstituted basement membrane preparations for use in accordance with the foregoing assay are readily available from numerous commercial suppliers. One suitable example membrane in this regard is "MATRIGEL" sold by Collaborative Biomedical Products of Bedford, MA.)

2. Quantitation

Invasive activity was measured by the number of cells that penetrated the RBM, as determined by a technique involving crystal violet staining developed

for use with the Boyden chamber. The technique, summarized below, is well known and is described, for instance, in Frandson *et al.*, *Fibrinolysis* 6(Supp4): 71-76 (1992), which is incorporated by reference herein in its entirety.

The RBM-coated membrane was removed from each chamber at the end of the incubation period. The filters were pinned down to a wax plate, keeping the surface with the invading cells upward. The cells were stained on the filters with 0.5% crystal violet in 25% methanol for 10 minutes. Then, the filters were rinsed in distilled water, four times or until crystal violet no longer leached into the wash water. After the wash, the surface of each filter that had been in contact with the wax plate was carefully wiped clean with a moist cotton swab, to remove nonmigrating cells. The filters then were placed in a 24-well cluster plate and dried overnight.

Crystal violet in the invading cells on each filter was extracted twice for 10 minutes into 500 µl aliquots of 0.1 M sodium citrate, 50% ethanol. The amount of crystal violet in the extract was analyzed by absorbance at 585 nm, using a standard spectrometer.

3. Analyses

Assays were carried out in triplicate for each data point.

Variance between control and test groups was analyzed for significance using the standard repeated measures test for analysis of variance. $P < 0.01$ generally was considered indicative of a significant effect, with exceptions, as noted elsewhere herein.

EXAMPLE 4 Correlation of optical density with cell counts, and assay of basal invasiveness

To calibrate optical density of the crystal violet extracts against the number of migrating cells

on filters, cells were counted, seeded at known densities on filters, incubated to allow attachment and washed. One set of a duplicate set of plates was used for cell counting. The other set was used for staining. For counting, cells were released from the filters by mild trypsinization and then counted using an automated cell counter. For staining, after washing, the cells were stained and crystal violet stain then was extracted from the stained cells, as described in EXAMPLE 3. The optical densities of the extracts were measured by standard spectroscopy. The optical density determined for each filter extract was matched with the number of cells attached to its companion filter, as determined by direct counting. These paired data points served to correlate optical density with cell counts. plotted that displayed the

In accordance with the foregoing procedure, several densities of DU-145 cells were seeded into the top chamber of the Boyden jars. The jars were set up in pairs, and for each pair dye uptake by the cells was measured on one filter and the number of cells was counted on the other filter, as described above.

The results are shown in Table I, which sets out the number of cells migrating to the lower face of the filters as a function of the number of cells seeded in the upper chamber. The number of cells migrating to the lower filter surface also is set out as a percentage of the total number of cells seeded in the top chamber. The data in the Table are the means of triplicate determinations for each condition, and the indicated variance is the standard error of the mean.

At cell seedings greater than 2×10^5 approximately 22% of cells invaded the RBM and migrated through the filter in 6 hr. Seeding higher numbers of cells did not increase invasiveness at 6 hr. (Not shown.)

The cells were counted using an automated cell counter, such as the "COULTER MULTISIZER" made by Coulter, Inc. of Hialeah, FL. By these experiments it was determined that an absorbance of 0.1 units of the crystal violet extract corresponds to approximately 5000 cells that migrated through the filter.

Similar procedures can be applied to calibrate the migration assay for other cells, to employ the assay to measure the therapeutic activity of other compositions of the present invention.

Table 1: Relationship between cell invasion and optical absorbance

Cells seeded ($\times 10^3$)	O.D. units[a] (585 nm)	Cells invading ($\times 10^1$)	Percentage invasion
100	0.60 ± 0.1	31.8 ± 0.2	31.8 ± 0.2
200	1.50 ± 0.2	42.0 ± 2.1	21.2 ± 1.0
300	1.20 ± 0.2	72.3 ± 2.7	23.0 ± 0.4

[a] 1 O.D.unit corresponds to approximately 5,000 cells.

As shown in Figure 1, cells of the TSU-Pr1 cell line exhibited the highest rate of basal invasiveness and the highest FCM-stimulated invasiveness. The basal rate for DU-145 cells was 2-fold lower than the rate for TSU-Pr1 cells, but the rate of stimulated invasiveness was comparable for the two cell lines. As shown in Figure 2, basal and stimulated invasiveness of PC3-M cells both were 2-fold lower than that observed for DU-145 cells. (*N.B.* The scale change in Figure 2.) LNCaP cells exhibited the lowest basal and the lowest stimulated invasiveness. FCM stimulation increased the invasiveness of this cell line 2-3-fold, also as shown in Figure 2.

EXAMPLE 5 Uteroglobin inhibits invasiveness of tumor cells

The ability of uteroglobin to inhibit tumor cell invasiveness is illustrated by its effect on cell lines treated with 0.01, 0.1 or 1.0 μM uteroglobin. Each of the four cells lines was incubated for 24 hr. with these concentrations of uteroglobin. After the incubation period the cells were rinsed and then assayed for invasiveness. Inhibition of stimulated

invasiveness then was quantitatively determined by subtracting the basal invasiveness from FCM-stimulated invasiveness, for each treatment group. Finally, the results were calculated as a percentage of the invasiveness of untreated control cells.

All four cell lines showed a dose-dependent inhibition of FCM-stimulated invasion, as shown in Figures 1 and 2, by the bars for FCM/UG. Notably, uteroglobin did not affect basal invasiveness, indicated by the bars labelled SFM/UG. Table 2 shows the average inhibition observed in three independent experiments, each of which was performed in triplicate. The inhibition of invasiveness by uteroglobin was found to be significant at the $P<0.01$ level for all conditions, except for PC3-M and TSU-Pr1 cells treated with 0.01 μM uteroglobin, which were significant at the $P<0.05$ level. As shown in Table 2, 1.0 μM uteroglobin inhibited invasiveness of DU-145 cells by 60%, PC3-M cells by 88%, LNCaP cells by 92% and the TSU-Pr1 cells by 59%.

Table 2: Inhibiti n of tumor cell invasiveness by ut roglobin

Cell lines	% Inhibition of invasion by uteroglobin 0.01 μM	0.1 μm	1.0 μM
DU-145	45.4 ± 6[a]	49.9 ± 5[a]	60.2 ± 11[a]
PC3-M	43.4 ± 15[b]	82.4 ± 14[a]	87.9 ± 11[a]
TSU-Pr1	33.5 ± 10[b]	44.4 ± 11[a]	58.9 ± 8[a]
LNCaP	71.5 ± 10[a]	81.3 ± 6[a]	92.3 ± 7[a]

The table shows that results of assays described herein above. Briefly, tumor cells were cultured in media containing uteroglobin for 24 hr. and then assayed for invasive activity. Basal migration was subtracted and the adjusted measure is expressed as percent of untreated control cells for each cell type. Data is expressed as mean of three independent experiments performed in triplicate, and the variance is the standard error of the mean.

[a] $P \leq 0.001$; *i.e.*, statistically significant at the 0.001 level.

[b] $P \leq 0.05$; *i.e.*, statistically significant at the 0.05 level.

EXAMPLE 6 Time course of inhibition of invasiveness by uteroglobin

The time course for suppression of invasiveness of DU-145 cells by uteroglobin was determined over a 24 hr. period. Cells were treated, as described above, for 3, 6, 12, or 24 hr. and then assayed for invasiveness. The maximum inhibition of invasiveness observed in the cells cultured in the presence of uteroglobin was 74%, at 12 hr. 50% maximum inhibition was observed after 3 hr., and at 24 hr. inhibition was 79% of the maximum. Figure 4 shows these results in graphical form.

EXAMPLE 7 Uteroglobin does not affect simple motility

Experiments carried out to assess motility *per se* show that uteroglobin does not affect normal cell motility, *i.e.*, migration in the absence of RBM. The

results show that uteroglobin specifically inhibits the invasion-associated motility of epithelial tumor cells. Invasion-associated motility, which is implicated more specifically in metastasis than motility *per se*, involves the synthesis, recruitment, or activation of several different classes of proteolytic enzymes including collagenases, cathepsins, plasminogen activators and a variety of metalloproteinases required for degradation of basement membranes and the ECM. The observation that uteroglobin does not alter cell motility, but inhibits FCM-stimulated invasiveness, indicates that uteroglobin specifically can inhibit metastatic invasiveness without directly altering motility of normal cells. This is an advantageous property for pharmacological intervention where non-specific effects of uteroglobin on normal motility could be disadvantageous.

EXAMPLE 8 Uterogl bin does not affect adhesion to RBM

The anti-invasive activity of uteroglobin is not mediated by an effect on cell adhesion. This can be seen from experiments in which the adhesiveness of DU-145 and PC3-M cells was measured after incubation for 24 hr. in SM media or SM media containing 1.0 μM uteroglobin for 24 hr. Adhesion was tested by removing the incubation media, resuspending the cells in fresh αMEM/SM, counting them, replating the cells and then counting the number of cells that had attached 1, 3 and 6 hr. after replating. The results show that uteroglobin does not alter adhesiveness of the cells. The absence of an effect of uteroglobin on basal migration, illustrated by the results depicted in Figures 1 and 2, supports the same conclusion. This is an advantageous property for pharmacological intervention where non-specific effects of uteroglobin on normal cell adhesiveness could be disadvantageous.

EXAMPLE 9 Specificity of uteroglobin anti-metastatic effects

The specificity of uteroglobin activity was demonstrated in DU-145 cells, using myoglobin, albumin and heat inactivated uteroglobin. Cells were treated for 24 hr with either myoglobin, albumin or uteroglobin that had been inactivated by incubation at 55°C for 45 min. The results, presented in the graph in Figure 3, show that myoglobin, albumin and heat-inactivated uteroglobin do not effect invasive activity of tumor cells.

EXAMPLE 10 Uteroglobin inhibits arachidonic acid release by FCM stimulated tumor cells

The effect of uteroglobin on the release of arachidonic acid ("AA") by tumor cells was assayed under conditions of basal and stimulated invasiveness. (^{14}C)AA having a specific activity 58.0 mCi/mmol was used to trace the arachidonic acid release. Labelled arachidonic acid of this type can be obtained from several commercial suppliers, such as Amersham, Inc. of Arlington Heights, IL.

Uteroglobin inhibits release of arachidonic acid by DU-145 cells stimulated by FCM, as shown by the following experiment.

Intracellular arachidonic acid in DU-145 cells was labeled by incubating the cells in media containing ^{14}C-labelled arachidonic acid. For this purpose approximately 0.75 X 10^5 cells were incubated for 24 hr. at 37°C in 2 ml of α-MEM/SF media containing 1μCi of (^{14}C)AA. After this, the cells were washed three times with 20 ml 0.2% bovine serum albumin to remove free radioactivity.

The washed, labelled cells were resuspended in 2 ml of α-MEM/SF, FCM or FCM containing 1.0 μM uteroglobin. Cells in each of the three media were incubated at 37°C and 50 μl aliquots were removed of the media were removed from each culture 0.5, 10, 20

and 30 min., and 1, 2, 3, 4 and 5 hr. after the beginning of the incubation period.

Each aliquot was assayed for AA release, which was measured as ^{14}C free in the media, determined by scintillation counting. Standard scintillents and counters were used to quantitate radiation emission by ^{14}C in the samples; *e.g.*, EcoLite Biodegradable scintillant from ICN, Inc.

Stimulation of AA release by FCM was calculated by subtracting the amount of (^{14}C)AA released by cells incubated in αMEM/SF media (which was very low) from the amount of (^{14}C)AA released by cells incubated in FCM media. The effect of uteroglobin on FCM-stimulated AA release was calculated in the same way, by subtracting the amount of (^{14}C)AA released by cells cultured in αMEM/SF from the amount of (^{14}C)AA released by cells incubated in FCM containing 1 μM uteroglobin.

As shown in the graph in Figure 5, arachidonic acid released by cells cultured in FCM media exhibited a biphasic profile. Released AA peaked at 20 min., the peak was followed by a period of reuptake, *e.g.* 60 min. and then there was a period of sustained release to the end of the 5 hr. incubation period of this experiment.

The presence of 1 μM uteroglobin in FCM media reduced FCM-stimulated release of arachidonic by 77% at 20 min. and 86% at 5 hr.

The dramatic inhibition of release of arachidonic acid by FCM-stimulated tumor cells, together with the foregoing results showing the inhibitory effect of uteroglobin on invasiveness, show that uteroglobin affects an early event in the signalling pathway(s) that control tumor invasiveness.

EXAMPLE 11

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears not to

have metastasized. The adenocarcinoma is removed by surgery. Uteroglobin is administered before and after surgery at a dose rate that reaches and then maintains a blood concentration of uteroglobin of approximately 1 μM. After post-operative recovery, the patient is maintained at a decreased level of uteroglobin by a regimen of periodic *i.m.* self-administration. No further occurrences of the adenocarcinoma develop.

EXAMPLE 12

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears not to have metastasized. The adenocarcinoma is removed by surgery. Uteroglobin is administered before and after surgery, at a dose rate that reaches and then maintains a blood concentration of uteroglobin of approximately 1 μM. After post-operative recovery, the patient is maintained at a decreased level of uteroglobin by intermittent or continuous administration by subdural pump. No further occurrences of the adenocarcinoma develop.

EXAMPLE 13

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears not to have metastasized. The adenocarcinoma is removed by surgery. Uteroglobin is administered before and after surgery, at a dose rate that reaches and then maintains a blood concentration of uteroglobin of approximately 1 μM. After post-operative recovery, the patient is maintained at a decreased level of uteroglobin by intermittent or continuous administration using a transdermal patch. No further occurrences of the adenocarcinoma develop.

EXAMPLE 14

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears to have metastasized, but surgery still is indicated as an effective treatment modality. Tumor tissue is removed by surgery. Uteroglobin is administered from the time, approximately, of the initial diagnosis and continues after surgery, *i.m.* and *i.v.*, at a dose rate that reaches and then maintains a blood concentration of uteroglobin above 1 μM. After post-operative recovery, the patient is maintained at this level of uteroglobin by a regimen of periodic *i.m.* self-administration. The patient is monitored carefully for intolerable adverse side-effects of high-dose uteroglobin administration. No further tumors develop.

EXAMPLE 15

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears to have metastasized, but surgery still is indicated as an effective treatment modality. Tumor tissue is removed by surgery. Uteroglobin is administered from the time, approximately, of the initial diagnosis and continues after surgery, *i.m.* and *i.v.*, at a dose rate that reaches and then maintains a blood concentration of uteroglobin above 1 μM. After post-operative recovery, the patient is maintained at this level of uteroglobin by a regimen of periodic *i.m.* self-administration. The patient is monitored carefully for intolerable adverse side-effects of high-dose uteroglobin administration. Although some of the original, small tumorous masses are detected after surgery, they do not grow in size.

EXAMPLE 16

A patient presents with metastatic adenocarcinoma of the prostate. The adenocarcinoma appears to have metastasized, but surgery still is indicated as an effective treatment modality. Tumor tissue is removed by surgery. Uteroglobin is administered from the time, approximately, of the initial diagnosis and continues after surgery, *i.m.* and *i.v.*, at a dose rate that reaches and then maintains a blood concentration of uteroglobin above 1 μM. After post-operative recovery, the patient is maintained at this level of uteroglobin by a regimen of periodic *i.m.* self-administration. The patient is monitored carefully for intolerable adverse side-effects of high-dose uteroglobin administration. Tumorous masses are detected after surgery, but their growth is slowed.

EXAMPLE 17

A patient presents with a tumor of the breast, of epithelial cell origin. The tumor, which appears to be of a metastatic type, appears not to have metastasized. The tumor is removed by surgery. Uteroglobin is administered after surgery, *i.m.* and *i.v.*, at a dose rate that reaches and then maintains a blood concentration of uteroglobin of approximately 1 μM. After post-operative recovery, the patient is maintained at a decreased level of uteroglobin by a regimen of periodic *i.m.* self-administration. No further occurrences of the tumor develop.

EXAMPLE 18

A patient presents with a breast tumor of epithelial cell origin. The breast tumor has metastasized. Numerous secondary tumors are detected. Insofar as possible, tumor tissue is removed by surgery. Surgical intervention is aggressive. Uteroglobin is administered from the time,

approximately, of the initial diagnosis and continues after surgery, *i.m.* and *i.v.*, at a dose rate that reaches and then maintains a blood concentration of uteroglobin above 1 μM. After post-operative recovery, the patient is maintained at this level of uteroglobin by a regimen of periodic *i.m.* self-administration. The patient is monitored carefully for intolerable adverse side-effects of high-dose uteroglobin administration. No further tumors develop in the remaining breast or elsewhere in the body.

EXAMPLE 19 Uteroglobin is not expressed or secreted by prostatic adenocarcinoma cells

A 76 year old male underwent radical perineal prostatectomy because of a biopsy-based diagnosis of prostatic adenocarcinoma with a Gleason's score of 4. Postsurgical definitive pathologic diagnosis showed moderately to poorly differentiated adenocarcinoma with a Gleason's score of 8, nodular prostatic hyperplasia, and multifocal high grade prostatic intraepithelial neoplasia (PIN). Perineural and lymphatic invasion was noted, but the seminal vesicles were free of tumor.

With informed consent and in accordance with approved procedures, prostatic tissue was obtained from the diseased prostate gland after removal, for evaluation of uteroglobin expression. Tissue fragments were frozen in liquid nitrogen. Individual slices of frozen tissue were sectioned by cryostat and mounted on silanated microscope slides.

Samples were fixed with 4% formalin for 3 minutes at room temperature and washed for 10 min in phosphate buffered saline (PBS) pH 8.0. Nonspecific reactivity was blocked by incubating samples with rabbit serum (1:100 dilution) for 30 min. The samples were then exposed to a 1:1,000 dilution of goat anti-human uteroglobin primary antibody overnight at 4 degrees C. Control samples were exposed to a 1:100 dilution of goat serum.

All samples were then exposed to biotinylated rabbit anti-goat antibody (1:10,000) for 30 min and then washed with PBS for 10 min. Streptavidin complex was added for 15 min and the samples washed again with PBS for 10 min. DAB was reacted with the samples for 2 min followed by standard staining of the tissue with hematoxylin-eosin.

Slides were analyzed by two certified pathologists, who were not informed of the identity of

any slides and who carried out their analyses independently.

In each case, the normal prostatic tissue stained strongly positive for uteroglobin in epithelial cells, especially at the intracellular luminal surface. Stromal cells were negative. Areas of hyperplasia diagnosed independently by both pathologists as PIN stained positively (PIN is considered by some to represent an early pre-neoplastic precursor). In contrast, tumorous epithelial cells exhibited little or no staining for uteroglobin, demonstrating that the tumor cells had lost the ability to synthesize and secrete this protein.

EXAMPLE 20 Uteroglobin mRNA is not detected or is aberrantly processed in cells derived from metastases of human prostatic tumors

RNA was isolated from normal prostate tissue and from the DU-145, PC-3 and TSU-PR1 cell lines derived from metastatic human prostate tumors. The RNA was subjected to Northern blotting according to routine and standard procedures, using a radiolabelled uteroglobin cDNA probe. Autoradiographic analysis showed that the normal tissue expresses an abundant normally processed 600 base pair mRNA uteroglobin transcript. In contrast the metastatic tumor cells either did not detectably express the uteroglobin transcript (DU-145 and PC-3) or expressed a grossly aberrant transcript (TSU-PR1).

EXAMPLE 21

A patient presents with urinary obstruction and after digital rectal exam a biopsy of the prostate is taken. The initial presurgical report assigns a Gleason's score of 3 suggesting a low-grade localized tumor. A portion of the biopsy sample is analyzed immunohistochemically for uteroglobin expression which is found to be present in the normal tissue but absent

in the tumor cells. The diagnosis, prognosis, and plan for therapy is appropriately altered to reflect the high probability, based on lack of uteroglobin expression, that the tumor is actually of higher grade than initially diagnosed and probably invasive and metastatic. Uteroglobin therapy is immediately begun.

EXAMPLE 22

After radical prostatectomy, a patient presents with high grade metastatic prostatic adenocarcinoma that has become refractory to hormonal therapy. The patient refuses chemotherapy based on its dismal efficacy against prostate cancer and its devastating side effects. In situ hybridization analysis of a tumor biopsy reveals that the uteroglobin gene is not being expressed in the tumor cells. Further analysis of uteroglobin gene structure by SSCP and RFLP indicate the that the gene is mutated and dysfunctional. The patient chooses to become a candidate for gene therapy. The patient is injected with an adenovirus-based plasmid expression vector containing the uteroglobin gene linked to the promotor of the PSA gene which is specifically expressed in prostate cells. The vector is encapsulated in liposomes which have anti-PSA antibody fixed on the surface. The antibody-liposome complex binds specifically to cells secreting PSA which presumably are only the metastatic tumor cells. The liposomes are ingested by the cells and release the plasmids which incorporate into the cells' genomic DNA and begin expressing uteroglobin. The transfected cells expressing uteroglobin reverse their invasive phenotype, thereby ceasing further metastasis and are gradually destroyed by the bodies natural defenses. The metastatic tumors regress and the patient's life is prolonged.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modification are intended to be included within the scope of the following claims.

What is claimed is:

1. A method for preventing or inhibiting metastasis of a cancer of epithelial cell origin, comprising the step of administering to an organism suffering from a cancer of epithelial cell origin a compound that inhibits arachidonic acid release by cells of said cancer, by a route and in an amount effective to inhibit or prevent metastasis of said tumor.

2. A method according to claim 1, wherein said compound is an inhibitor of phospholipase A_2 or cyclooxygenase.

3. A method according to claim 1, wherein said compound is mepacrine or indomethacin.

4. A method according to claim 1, wherein said compound is a lipocortin, a mutein of a lipocortin, a peptide analog of a lipocortin, or a mimetic of lipocortin.

5. A method according to claim 1, wherein said compound is a uteroglobin, a mutein of a uteroglobin, a peptide analog of a uteroglobin or a mimetic of uteroglobin.

6. A method according to claim 1, wherein said compound is a uteroglobin.

7. A method according to claim 6, wherein said compound is human uteroglobin.

8. A method according to claim 1, wherein said tumor is a cancer of the prostate gland in a human patient.

9. A method according to claim 2, wherein said tumor is a cancer of the prostate gland in a human patient.

10. A method according to claim 5, wherein said tumor is a cancer of the prostate gland in a human patient.

11. A method according to claim 6, wherein said tumor is a cancer of the prostate gland in a human patient.

12. A method according to claim 7, wherein said tumor is a cancer of the prostate gland in a human patient.

13. A method according to claim 1, wherein said method is used in conjunction with another treatment.

14. A method according to claim 13, wherein said treatment is surgical intervention, radiation therapy, hormonal therapy, immunotherapy, chemotherapy, cryotherapy or gene therapy.

15. A pharmaceutical composition for inhibiting or preventing metastasis of a cancer of epithelial cell origin, comprising: (i) a compound that inhibits arachidonic acid release by cells of a tumor of epithelial cell origin effective to prevent or inhibit metastasis of said tumor in an organism and (ii) a carrier for effective therapeutic administration of said compound to said organism.

16. A composition according to claim 15, wherein said compound is an inhibitor of phospholipase A_2 or cyclooxygenase.

17. A composition according to claim 15, wherein said compound is mepacrine or indomethacin.

18. A composition according to claim 17, wherein said compound is a lipocortin, a mutein of a lipocortin, a peptide analog of a lipocortin or a mimetic of lipocortin.

19. A composition according to claim 15, wherein said compound is a uteroglobin, a mutein of a uteroglobin a peptide analog of a uteroglobin or a mimetic of uteroglobin.

20. A composition according to claim 19, wherein said compound is a uteroglobin.

21. A composition according to claim 20, wherein said compound is human uteroglobin.

22. A method for determining metastatic potential of a tumor of epithelial cell origin, comprising the steps of:

(A) determining an effector of arachidonic acid release in cells in a biopsy sample of a tumor;

(B) comparing effector in tumor cells in said biopsy sample with effector in fiduciary cells, and

(C) determining metastatic potential, wherein effector in said tumor cells characteristic of normal fiduciary cells or characteristic of fiduciary cells of benign tumors indicates low metastatic potential and effector in said tumor cells characteristic of fiduciary cells of metastatic tumors indicates high metastatic potential.

23. A method for determining metastatic potential of a tumor, according to claim 22, wherein said effector is an inhibitor of PLA_2 or cyclooxygenase.

24. A method for determining metastatic potential of a tumor according to claim 23, wherein said inhibitor is uteroglobin.

25. A method according to claim 22, wherein said effector is determined assaying a protein in cells of said tumor.

26. A method for determining metastatic potential of a tumor, according to claim 25, wherein said effector is an inhibitor of PLA_2.

27. A method for determining metastatic potential of a tumor according to claim 26, wherein said inhibitor is uteroglobin.

28. A method according to claim 25, wherein said protein is determined by immunocytochemistry.

29. A method for determining metastatic potential of a tumor according to claim 28, wherein said effector is an inhibitor of PLA_2.

30. A method for determining metastatic potential of a tumor according to claim 29, wherein said inhibitor is uteroglobin.

31. A method according to claim 22, wherein said effector is determined by assaying an mRNA in cells of said tumor.

32. A method for determining metastatic potential of a tumor according to claim 31, wherein said effector is an inhibitor of PLA_2.

33. A method for determining metastatic potential of a tumor according to claim 32, wherein said inhibitor is uteroglobin.

34. A method according to claim 31, wherein said mRNA is determined by *in situ* hybridization.

35. A method for determining metastatic potential of a tumor, according to claim 34, wherein said effector is an inhibitor of PLA_2.

36. A method for determining metastatic potential of a tumor according to claim 35, wherein said inhibitor is uteroglobin.

37. A method according to claim 32, wherein an aberrant mRNA is determined.

38. A method for determining metastatic potential of a tumor according to claim 37, wherein said inhibitor is uteroglobin.

39. A method according to claim 25, wherein said tumor is a prostatic adenocarcinoma, and said inhibitor is uteroglobin.

40. A method according to claim 31, wherein said tumor is a prostatic adenocarcinoma, and said inhibitor is uteroglobin.

41. A kit for determining metastatic potential of a tumor, comprising:

(A) a first reagent that binds specifically to an effector of arachidonic acid release in cells in a biopsy sample prepared for determination of said effector, and

(B) a second reagent for detectably labelling said primary binding reagent bound specifically to cells in said biopsy sample,

wherein the determination of said effector tumor is diagnostic of the metastatic potential of said tumor.

42. A kit according to claim 41, wherein said effector is an inhibitor of PLA_2.

43. A kit according to claim 42, wherein said inhibitor is uteroglobin.

44. A kit according to claim 41, wherein said first reagent is an antibody.

45. A kit according to claim 44, wherein said effector is an inhibitor of PLA_2.

46. A kit according to claim 45, wherein said inhibitor is uteroglobin.

47. A kit according to claim 41, wherein said first reagent is a hybridization probe.

48. A kit according to claim 47, wherein said effector is an inhibitor of PLA_2.

49. A kit according to claim 48, wherein said inhibitor is uteroglobin.

50. A method for identifying prostatic intraepithelial neoplasia, comprising the steps of:

(A) determining an effector of arachidonic acid release in cells in a biopsy sample of tissue;

(B) comparing effector in cells in said biopsy sample with effector in fiduciary cells, and

(C) identifying presence of prostatic intraepithelial neoplasia, wherein a high concentration of effector in said biopsy sample indicates normal tissue or low grade prostatic intraepithelial neoplasia, a low concentration of effector in said biopsy sample indicates high grade prostatic intraepithelial neoplasia, and substantially no concentration of effector in said biopsy sample indicates cancer.

51. A method for identifying prostatic intraepithelial neoplasia according to claim 50, wherein said effector is an inhibitor of PLA_2 or cyclooxygenase.

52. A method for identifying prostatic intraepithelial neoplasia according to claim 51, wherein said inhibitor is uteroglobin.

53. A method according to claim 50, wherein said effector is determined by assaying a protein in cells of said biopsy sample.

54. A method for identifying prostatic intraepithelial neoplasia according to claim 53, wherein said effector is an inhibitor of PLA_2.

55. A method for identifying prostatic intraepithelial neoplasia according to claim 54, wherein said inhibitor is uteroglobin.

56. A method according to claim 53, wherein said protein is determined by immunocytochemistry.

57. A method for identifying prostatic intraepithelial neoplasia according to claim 56, wherein said effector is an inhibitor of PLA_2.

58. A method for identifying prostatic intraepithelial neoplasia according to claim 57, wherein said inhibitor is uteroglobin.

59. A method according to claim 50, wherein said effector is determined by assaying an mRNA in cells of said biopsy sample.

60. A method for identifying prostatic intraepithelial neoplasia according to claim 59, wherein said effector is an inhibitor of PLA_2.

61. A method for identifying prostatic intraepithelial neoplasia according to claim 60, wherein said inhibitor is uteroglobin.

62. A method according to claim 59, wherein said mRNA is determined by *in situ* hybridization.

63. A method for identifying prostatic intraepithelial neoplasia according to claim 62, wherein said effector is an inhibitor of PLA_2.

64. A method for identifying prostatic intraepithelial neoplasia according to claim 63, wherein said inhibitor is uteroglobin.

65. A kit for identifying prostatic intraepithelial neoplasia, which comprises:

(A) a first reagent that binds specifically to an effector of arachidonic acid release in cells in a biopsy sample prepared for identification of said effector, and

(B) a second reagent for detectably labelling said primary binding reagent bound specifically to cells in said biopsy sample,

wherein the identification of said effector is diagnostic of said prostatic intraepithelial neoplasia.

66. A kit according to claim 65, wherein said effector is an inhibitor of PLA_2.

67. A kit according to claim 66, wherein said inhibitor is uteroglobin.

68. A kit according to claim 65, wherein said first reagent is an antibody.

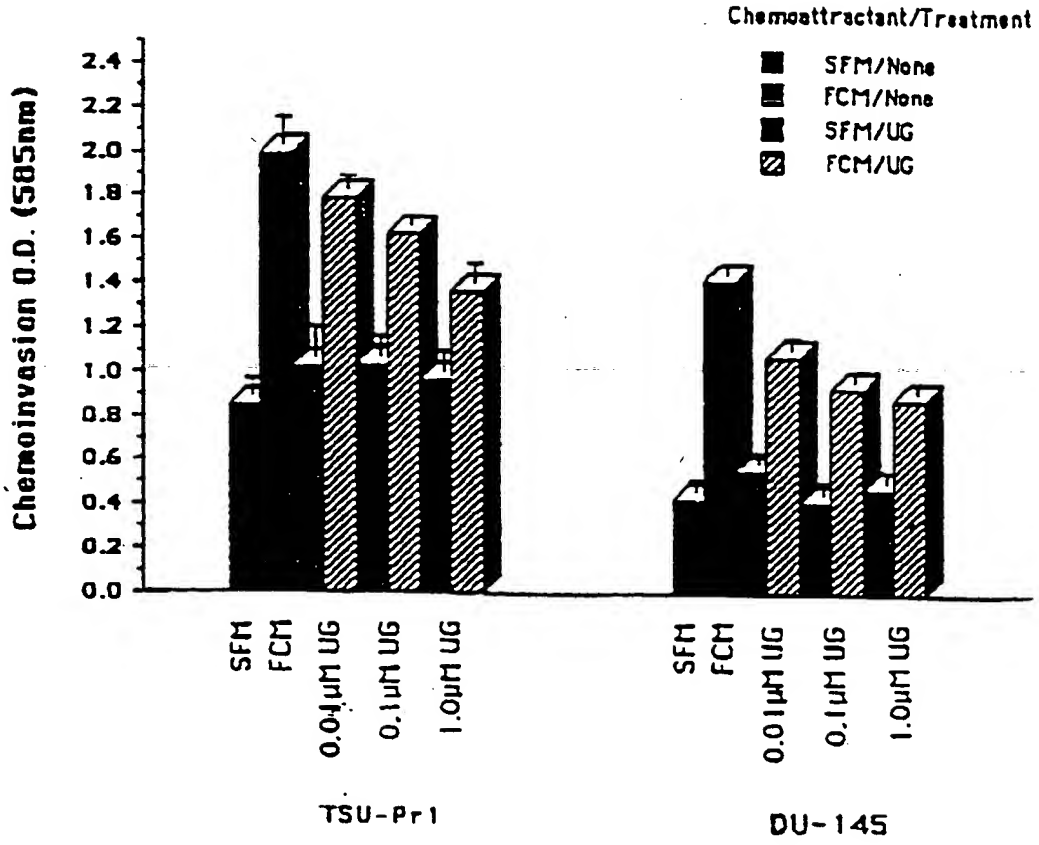
69. A kit according to claim 68, wherein said effector is an inhibitor of PLA_2.

70. A kit according to claim 69, wherein said inhibitor is uteroglobin.

71. A kit according to claim 65, wherein said first reagent is a hybridization probe.

72. A kit according to claim 71, wherein said effector is an inhibitor of PLA_2.

73. A kit according to claim 72, wherein said inhibitor is uteroglobin.


Chemoattractant/Treatment
SFM/None
FCM/None
SFM/UG
FCM/UG
Chemoinvasion O.D. (585nm)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
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FCM
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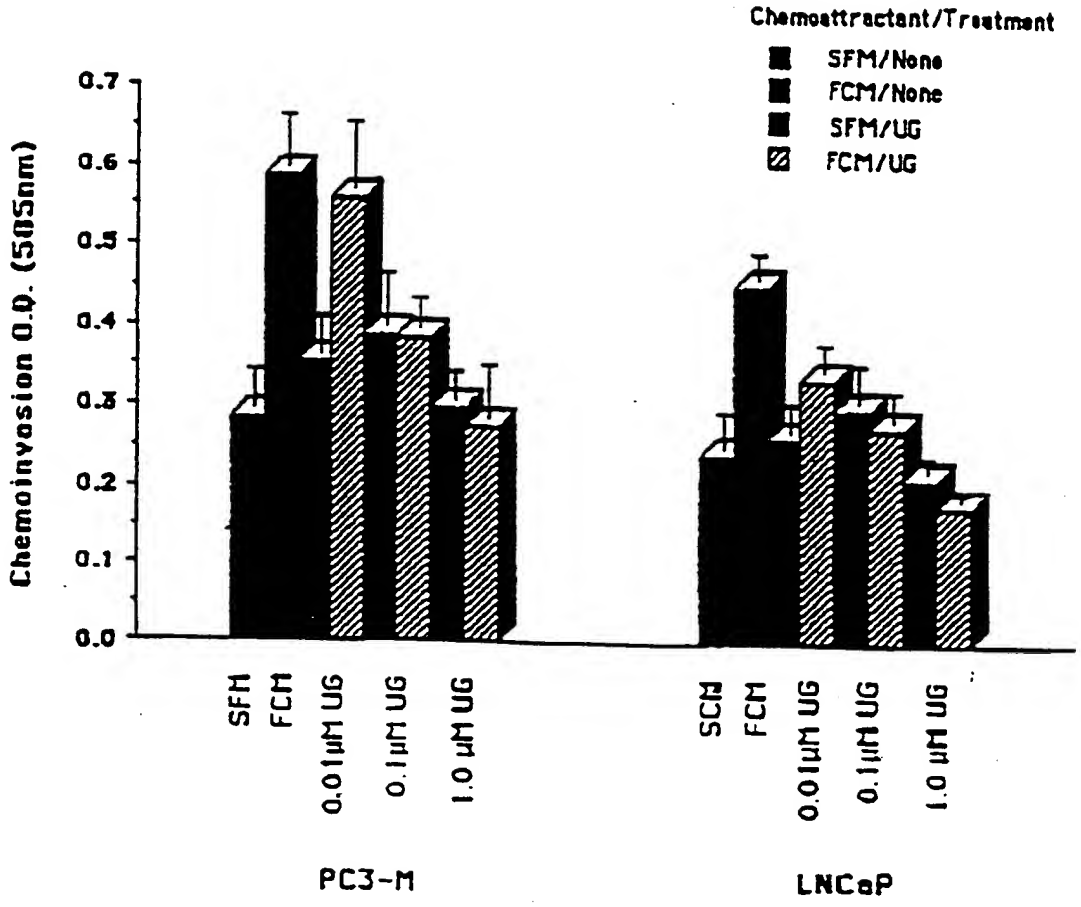
1.0μM UG
TSU-Pr1
DU-145

FIGURE 1


Chemoattractant/Treatment
SFM/None
FCM/None
SFM/UG
FCM/UG
Chemoinvasion O.D. (585nm)
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0.6
0.5
0.4
0.3
0.2
0.1
0.0
SFM
FCM
0.01μM UG
0.1μM UG
1.0 μM UG
SCM
FCM
0.01μM UG
0.1μM UG
1.0 μM UG
PC3-M
LNCaP

FIGURE 2

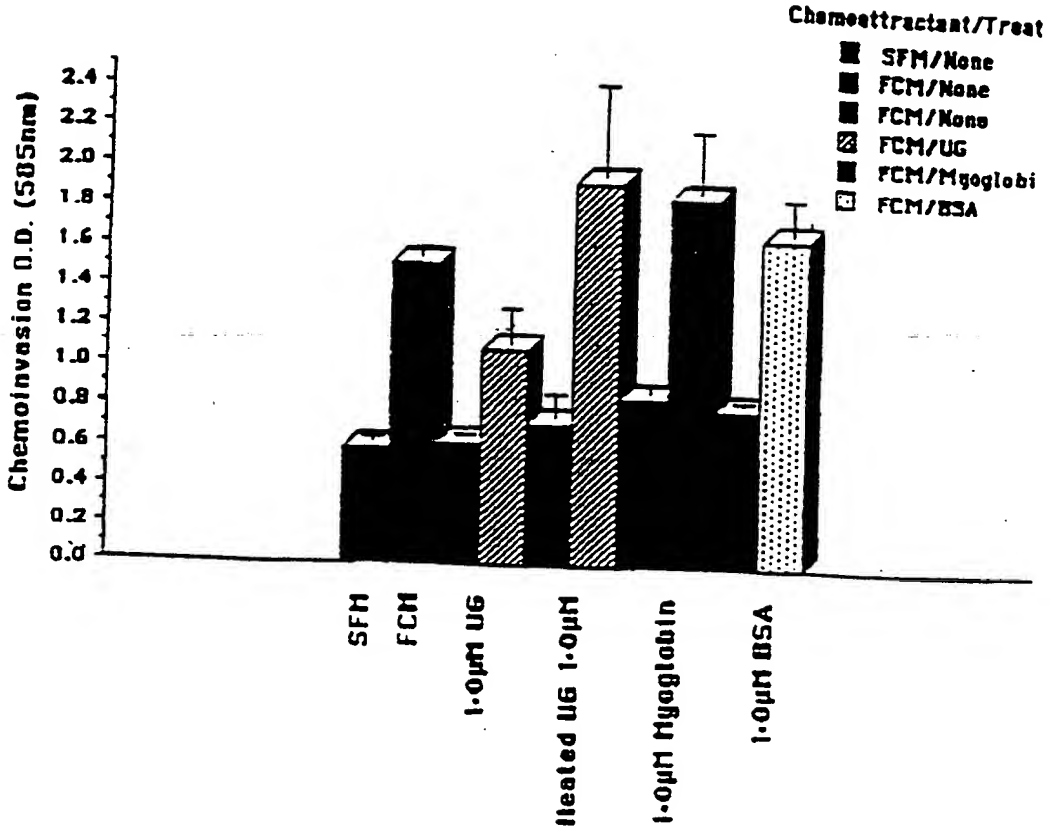
Chemoattractant/Treat
SFM/None
FCM/None
FCM/None
FCM/UG
FCM/Myoglobi
FCM/BSA
Chemoinvasion O.D. (585nm)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SFM
FCM
1.0μM UG
Heated UG 1.0μM
1.0μM Myoglobin
1.0μM BSA

FIGURE 3

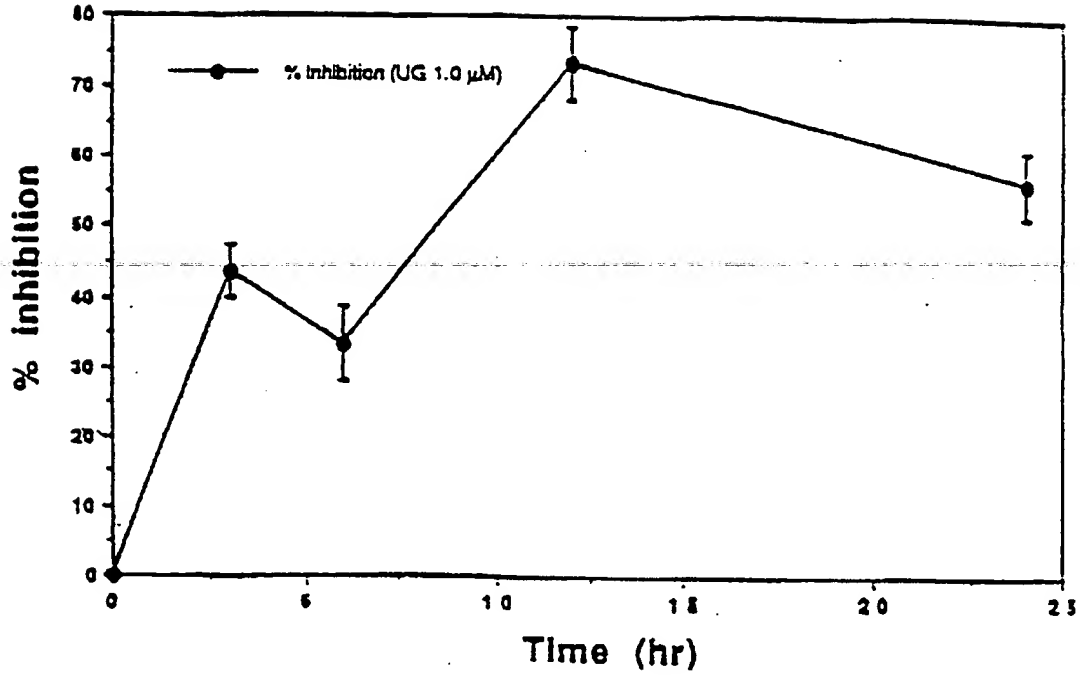
% Inhibition (UG 1.0 µM)
% Inhibition
Time (hr)
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25

FIGURE 4

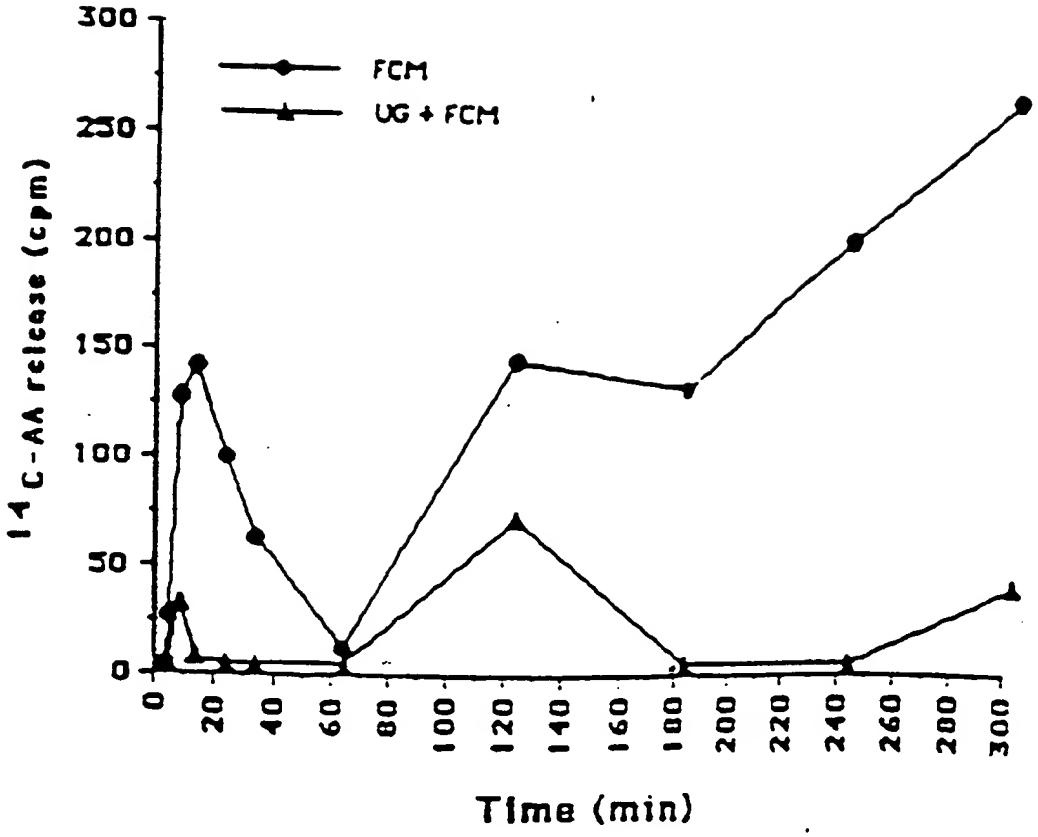
300
250
200
150
100
50
0
FCM
UG + FCM
^{14}C-AA release (cpm)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Time (min)

FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10221

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07D 249/06; C12N 5/08; A61K 31/41
US CL :548/255; 544/238; 514/359; 436/510; 435/240
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 548/255; 544/238; 514/359; 436/510; 435/240

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,489,166 (JOSHI) 18 December 1984, see entire document.	1-73
Y	US, A, 5,132,315 (KOHN ET AL.) 21 July, 1992, see column 1, line 59 to column 2, line 16.	1-73
Y	US, A, 5,219,752 (TAKAZAWA ET AL.) 15 June 1993, see column 12, lines 52-63.	1-73
Y	US, A, 5,359,078 (KOHN ET AL.) 25 October 1994, see entire document.	1-73
A, P	US, A, 5,482,954 (KOHN ET AL.) 09 January 1996, see entire document.	1-73

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
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Published
With international search report.

(54) Title: PHOSPHOLIPASE INHIBITORS FOR THE TREATMENT OF CANCER

(57) Abstract

The present invention relates generally to a method of treating disease conditions by the administration of an inhibitor of phospholipase activity. More particularly, the present invention contemplates a method for facilitating apoptosis of cancer cells or otherwise reducing or preventing growth of cancer cells by inhibiting phospholipase activity. Even more particularly, the present invention contemplates the use of inhibitors of phospholipase A_2 enzymes in the treatment and prophylaxis of cancer. The present invention further provides biological compositions comprising an inhibitor of phospholipase A_2 alone or in combination with other agents in the treatment of cancer.

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PHOSPHOLIPASE INHIBITORS FOR THE TREATMENT OF CANCER

FIELD OF THE INVENTION

The present invention relates generally to a method of treating disease conditions by the administration of an inhibitor of phospholipase activity. More particularly, the present invention contemplates a method for facilitating apoptosis of cancer cells or otherwise reducing or preventing growth of cancer cells by inhibiting phospholipase activity. Even more particularly, the present invention contemplates the use of inhibitors of phospholipase A_2 enzymes in the treatment and prophylaxis of cancer. The present invention further provides biological compositions comprising an inhibitor of phospholipase A_2 alone or in combination with other agents in the treatment of cancer.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

The increasing sophistication of diagnostic and surgical techniques is greatly facilitating the treatment of cancer. However, despite the improvements in the diagnosis and surgical treatment of cancer, the development of efficacious yet non-harmful anti-cancer agents has been slow.

Cancer is a most serious and debilitating disease condition facing both the human and animal populations. The term "cancer" covers a range of malignant cell conditions and encompassing relatively minor conditions as well as serious and generally fatal conditions. For example, gastric cancer is a major contributor of cancer-related deaths throughout the world. According to the World Health Organisation (1), in 1993, gastric cancer was the fourth leading cause of cancer death in the United States and the second leading cause of cancer death in Japan. Colorectal cancer is the second leading cause of cancer death in the

United States (2). However, despite a greater understanding of the genetic bases of this type of cancer, non-surgical treatment of colorectal cancer has not been overly successful.

Recent reports have provided evidence for a role for aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) in reducing cancer development (3-7). However, prolonged use of these compounds can lead to adverse gastrointestinal side-effects. One common target for NSAIDs is the enzyme cycloxygenase. This enzyme exists in two isoforms, referred to herein as "COX1" and "COX2". Most NSAIDs do not discriminate between COX1 and COX2 (8-10). COX1 is constitutively expressed in a number of cells (11) whereas COX2 is inducible by, for example, growth factors and cytokines (12, 13). It is apparent, therefore, that COX2 gene expression is elevated in inflammatory cells and sites of inflammation.

COX1 and COX2 play important roles in physiological processes such as prostaglandin biosynthesis. The latter is important since excessive prostaglandin production is implicated and associated with proinflammatory eicosanoid, inhibition of production of immune regulatory lymphokines, inhibition of T- and B-cell proliferation, inhibition of cytotoxic activity of natural killer cells, induction of immunosuppression-facilitating molecules (e.g. TNF and IL-10) and reduced apoptosis of colon cancer cells.

Another important component of the regulatory pathway to prostaglandin biosynthesis is phospholipase and, in particular, phospholipase A_2 (hereinafter referred to as "PLA_2").

During prostaglandin biosynthesis, membrane phospholipids are metabolised by phospholipases. Phospholipases are carboxylic acid esterases classified as phospholipase (PL) A_1, A_2, B and the phosphodiesterases, which are specific for lecithins. PLA_2 removes the unsaturated fatty acid at the C-2 of glycerol. The product of PLA_2 activity is arachidonic acid which is then catalytically converted to prostaglandin *via* the COX enzymes.

The inventors have surprisingly discovered that phospholipase inhibitors which target PLA_2 are useful for modulating cancer growth and development

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence Identity Numbers for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

The subject specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. <400>1, <400>2, etc).

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other

than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

One aspect of the present invention contemplates a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof.

More particularly, the present invention contemplates a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof which phospholipase inhibitor or derivative or homologue reduces the levels and/or activities of a phopholipase to an extent to reduce the growth and/or development of cancer cells.

Another aspect of the present invention provides a method for reducing the volume of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof which phospholipase inhibitor or a derivative or homologue reduces the levels and/or activities of a phopholipase to an extent to reduce the growth and/or development of cancer cells.

A further aspect of the present invention contemplates a method for controlling the growth and/or development of a cancer or the volume of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor or a functional derivative or homologue thereof which PLA_2 inhibitor or a derivative or homologue reduces the levels and/or activities of one or more types of PLA_2 to an extent to reduce the growth and/or development and/or volume of the cancer.

Yet a further aspect of the present invention contemplates a method for controlling the

growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor having an amino acid sequence substantially as set forth in any one or more of SEQ ID NOs: 1 to 11 or SEQ ID Nos: 12 to 33 or an amino acid sequence having at least 60% identity to any one or more of SEQ ID NOs: 1 to 11 or 12 to 33 or a functional derivative or homologue thereof which PLA_2 inhibitor or derivative or homologue reduces the level or activity of secretory PLA_2.

Yet another further aspect of the present invention provides a biological composition useful for the treatment and/or prophylaxis of cancer in a target animal or bird such as a human, primate, livestock animal or companion animal said composition comprising a PLA_2 inhibitor such as but not limited to the PLA_2 defined by any one of amino acids sequences set forth in SEQ ID NOs: 1-11 or 12 to 33 or a derivative, homologue, analogue or functional equivalent thereof.

Another aspect provides an agent for use in treating or preventing cancer, said agent comprising a PLA_2 inhibitor or a functional derivative, homologue or analogue thereof.

Yet another aspect of the present invention contemplates the use of a PLA_2 inhibitor or functional derivative, homologue or analogue in the manufacture of a medicament for the treatment or prophylaxis of cancer in an animal (e.g. human) or bird.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of the interaction between extracellular, membrane associated and cytosolic factors in the production of prostaglandins.

Figure 2 is a graphical representation of the effects of NSI inhibitor on BGC-823 cancer growth in nude mice following subcutaneous administration.

Figure 3 is a graphical representation of the effects of NSI inhibitor on BGC-823 cancer growth in nude mice following intraperitoneal administration.

Figure 4 is a graphical representation of the effects of NS398 inhibitor on BGC-823 cancer growth in nude mice following subcutaneous administration.

Figure 5 is graphical representation of the effects of NS398 inhibitor on BGC-823 cancer growth in nude mice following intraperitoneal administration.

Figure 6 is a graphical representation of the effects of NSI plus NS398 inhibitors on BGC-823 cancer growth in nude mice following subcutaneous administration.

Figure 7 is a graphical representation of the effects of NSI plus NS398 inhibitors on BGC-823 cancer growth in nude mice following intraperitoneal administration.

Figure 8 is a graphical representation of the growth of BGC-823 and SGC-7901 cancers in nude mice.

Figure 9 is a graphical representation of the effects of NSI plus NS398 inhibitors on BGC-823 cancer growth in nude mice following subcutaneous administration.

Figure 10 is a graphical representation of the combined effects of NSI plus NS398 inhibitors on BGC-823 cancer growth in nude mice following subcutaneous administration.

Figure 11 is a graphical representation of the effects of NSI and NS398 inhibitors on BGC-823 cancer growth in nude mice following intraperitoneal administration.

Figure 12 is a graphical representation of the combined effects of NSI plus NS398 inhibitors on BGC-823 cancer growth in nude mice following intraperitoneal administration.

Figure 13A is a graphical representation showing the inhibition of non-snake venom PLA_2 by NSI, dilution group 1.

Figure 13B is a graphical representation showing the inhibition of non-snake venom PLA_2's by NSI, dilution group 2.

Figure 14A is a graphical representation showing the inhibition of snake venom PLA_2 enzymes with NSI, Day 1.

Figure 14B is a graphical representation showing the inhibition of snake venom PLA_2 enzymes with NSI, Day 2.

Figure 15 is a graphical representation showing the inhibition of $rhPLA_2$ by NSI.

A summary of SEQ ID Nos used herein is given in Table 1.

TABLE 1

SUMMARY OF SEQ ID NOS:

SEQ ID NO	DESCRIPTION
1	Amino acid sequence of NS1 ∝-chain including leader sequence
2	Amino acid sequence of *Oxyuranus scutellatus* PLA_2 inhibitor ∝-chain
3	Nucleotide and amino acid sequences of *Oxyuranus microlepidotus* PLA_2 inhibitor ∝-chain
4-11	Amino acid sequence of tryptic peptides of NSI β-chain
12	N-terminal amino acid sequence of ∝-chain isoform 1 of NAI
13	N-terminal amino acid sequence of ∝-chain isoform 2 of NAI
14-18	Enzymatic digest of mature α-chain of NAI
19	N-terminal amino acid sequence of β-chain of NAI
20-29	Enzymatic digest of mature β-chain of NAI
30-32	Amino acid sequence of mature ∝-chain determined from DNA sequence (minus leader sequence) of NAI
33	Amino acid sequence of mature β-chain of NAI
34	Nucleotide sequence encoding SEQ ID NO:30 (mature ∝-chain minus leader sequence)
35	Nucleotide sequence encoding SEQ ID NO:31 (mature ∝-chain minus leader sequence)
36	Nucleotide sequence encoding SEQ ID NO:32 (mature ∝-chain minus leader sequence)
37	Nucleotide sequence encoding SEQ ID NO:33 (mature β-chain minus leader sequence)
38-40	Amino acid sequence of leader sequence of ∝-chain of NAI determined from nucleotide sequence
41	Amino acid sequence of leader sequence of β-chain of NAI determined from nucleotide sequence
42	Nucleotide sequence of SEQ ID NO:38 (leader sequence of ∝-chain of NAI)
43	Nucleotide sequence of SEQ ID NO:39 (leader sequence of ∝-chain of NAI)

SEQ ID NO	DESCRIPTION
44	Nucleotide sequence of SEQ ID NO:40 (leader sequence of ∝-chain of NAI)
45	Nucleotide sequence of SEQ ID NO:41 (leader sequence from β-chain of NAI)

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, the inventors have determined that an inhibitor of PLA_2 and in particular secretory PLA_2 ($sPLA_2$) is effective in controlling the growth and development of cancer.

Accordingly, one aspect of the present invention contemplates a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof.

More particularly, a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof which phospholipase inhibitor or derivative or homologue reduces the levels and/or activities of a phopholipase to an extent to reduce the growth and/or development of cancer cells.

The present invention is particularly directed to the treatment and prophylaxis of cancers in animals such as humans, primates, livestock animals (e.g. sheep, goats, horses, cows, donkeys) laboratory test animals (e.g. mice, rats, guinea pigs, rabbits, hamsters), companion animals (e.g. dogs, cats) and captive wild animals. The present invention also extends, however, to avain species such as but not limited to poultry birds (e.g. chickens, geese, ducks, turkeys), game birds (e.g. pheasant, wild ducks, peacocks, emus, ostriches) and caged birds. The preferred targets for cancer therapy are animals such as humans, primates and laboratory test animals. More preferably, the target is human.

Reference to "controlling the growth and/or development" of cancer includes the induction of apoptosis and/or necrosis in cancer cells as well as reducing, inhibiting or otherwise retarding growth of cancer cells or the risk of cancer cell development. An analysis of the

effects on cancer cell growth may be conducted by any means but is conveniently determined by the "volume" of cancer cell material. The term "controlling the growth and/or development" of cancer includes, therefore, controlling the volume of a cancer as well as reducing, inhibiting or otherwise retarding the volume of a cancer.

Assessment of cancer cell death or apoptosis may be made by any convenient means such as but not limited to macroscopic examination, microscopic examination, the determination of metaphase frequency, the determination of the proportion of cells in the S-phase, examination of cell lysis, determination of nuclear damage, an analysis of nuclear fragmentation and/or a determination of the percentage of cells with subdiploid DNA.

Accordingly, another aspect of the present invention provides a method for reducing the volume of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof which phospholipase inhibitor or a derivative or homologue reduces the levels and/or activities of a phopholipase to an extent to reduce the growth and/or development of cancer cells.

The term "cancer" is used in its broadest sense and includes benign and malignant leukemias, sarcomas and carcinomas. The cancers contemplated by the present invention may be simple (i.e. composed of a single neoplastic cell type), mixed (i.e. composed of more than one neoplastic cell type) or compound (i.e. composed of more than one neoplastic cell type and derived from more than one germ layer). Examples of simple cancers encompassed by the present invention include tumours of mesenchymal origin (e.g. tumours of connective tissue, endothelial tissue, blood cells, muscle cells) and tumours of epithelial origin. Particular cancers contemplated by the present invention include fibrosarcoma, myxosarcoma, Ewing's sarcoma, granulocytic leukemia, basal cell carcinoma, colon cancer, gastric cancer and a variety of skin cancers.

The preferred phospholipase inhibitors of the present invention are those which inhibit

PLA_2.

Even more preferably, the phospholipase inhibitor inhibits more than one type of PLA_2 molecule.

PLA_2 enzymes comprise several sub-types, for example human Type I PLA_2 which is derived from human pancreas (14, 15) and human type II which is derived from human synovium, amongst others. Another PLA_2 enzyme is type V PLA_2 (16).

Accordingly, another aspect of the present invention contemplates a method for controlling the growth and/or development of a cancer or the volume of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor or a functional derivative or homologue thereof which PLA_2 inhibitor or a derivative or homologue reduces the levels and/or activities of one or more types of PLA_2 to an extent to reduce the growth and/or development and/or volume of the cancer.

Preferably, the PLA_2 inhibitor inhibits more than one type of PLA_2 molecule.

Preferably, the PLA_2 inhibitor is in isolated form and may be a proteinaceous molecule, lipid and/or polysaccharide or may be in another chemical form.

The term "isolated" means that the PLA_2 inhibitor of the present invention is provided in a form which is distinct from that which occurs in nature, preferably wherein one or more contaminants have been removed. Accordingly, the isolated PLA_2 inhibitor may be used in partially-purified or substantially pure form, in which a substantial amount of contaminants have been removed and/or is in a sequencably pure or substantially homogeneous form.

The term "sequencably pure" means that the isolated PLA_2 inhibitor is provided in a form

which is sufficiently purified to facilitate amino acid sequence determination using procedures known to those skilled in the art.

The term "substantially homogeneous" means that the isolated PLA_2 is at least about 75% free of contaminants, more preferably at least about 80% free of contaminants, including 90-100% purity.

The preferred phospholipase inhibitor in accordance with the present invention is one which is derivable from the serum or other bodily fluid of a venomous animal such as a venomous insect or venomous snake, amongst others.

PLA_2 inhibitors useful in the practice of the present invention is from the Australian tiger snake *Notechis scutatus* or the Tasmanian tiger snake *Notechis ater*. The present invention extends, however, to PLA_2 inhibitors from the serum or bodily fluid from a range of other venomous animals including a range of venomous snakes. The present invention extends to PLA_2 inhibitors identified following natural product screening from, for example, plants, microorganisms, river and sea beds and aquatic and antarctic environments.

Examples of insects, snakes and aquatic animals from which a PLA_2 inhibitor may be isolated include arachnids (eg. spiders, scorpions, mites, etc) insects (eg. wasps, bees, ants, fleas, etc), reptiles (eg. snakes, lizards, etc), amphibians (eg. toads, frogs) or aquatic animals (eg: fish, cephalopods, box jellyfish, Portuguese man-of-war jellyfish, blue-ringed octopus, etc), amongst others.

Examples of snakes include snakes from the family Colubridae (colubrid snakes such as species of the genera *Heterodon, Natrix, Regina, Clonophis, Thamnophis, Lampropeltis, Opheopdris, Coluber, Masticophis, Drymobius, Salvadora, Phyllorhyncus, Elaphe, Hydrodunastes, Ptyas, Calamaria, Lycodon, Mehelya, Boaedon, Farancia, Fordonia, Erpeton,* amongst others), Elapidae (cobras such as species of the genera *Ophiophagus, Naja, Oxyuranus, Pseudohaje, Walterinnesia, Aspidelaps, Boulengerina, Dendroaspis,*

Bungaris, Calliophis, Maticora, Micurus, Micruroides, Acanthophis, Notechis and *Australaps*, amongst others), Hydrophiidae (sea snakes such as species of the genera *Laticauda, Aipysurus, Hydrophis* and *Enhydrina*, amongst others), Viperidae (vipers, such as species of the genera *Viptera, Echis, Cerastes, Bitis, Atractaspis* and *Causus,* amongst others) and Crotalidae(pit vipers such as species of the genera *Crotalis, Sistrurus, Bothrops, Trimeresurus, Lachesus* and *Agkistrodon,* amongst others).

Particularly preferred snakes include snakes from the family Viperidae, such as *Viptera* spp. and *Bitis* spp., in particular, *V. russelli, A. bilineatus* and *B. alternatus* ; the family Crotalidae, such as the moccasin snakes and vipers (*Agkistrodon* spp.) and the rattlesnakes (*Crotalus* spp.), in particular *Crotalus atrox*; or the family Elapidae, such as but not limited to King cobra *(Ophiohagus hannah);* True cobras *(Naja* spp); Asian or Indian cobra *(N. naja);* Egyptian cobra *(N. haje);* Spitting cobra *(N. nigricolli);* Black-lipped cobra *(N. malenoleuca);* Cape cobra *(N. nivea);* Gold's tree cobra *(Pseudohaje goldii);* Desert black snakes *(Walterinnesia* spp); Shield-nose snakes *(Aspidelaps* spp); Water cobras or water snakes *(Boulengerina* spp); Black mamba *(Dendroaspis polylepis);* Mamba *(D. angusticeps);* Kraits snake *(Bungarus* spp); Oriential coral snakes *(Calliophis* spp); Long-glanded coral snakes *(Maticora* spp); American coral snakes *(Micurus* spp); Southern coral snake *(M. frontalis);* Eastern coral snake or Harlequin snake *(M. fulvius);* Western coral snake *(Micruroides* spp); Arizona coral snake *(M. euryxanthus);* Death adder *(Acanthophis antarcticus);* Australian tiger snakes *(Notechis* spp e.g. *N. scutatus* or *N. ater);* and Australian copperhead *(Australaps* spp), amongst others.

The present inventors have determined that the *N. scutatus* and *N. ater* PLA_2 inhibitors inhibit more than one type of PLA_2 and in particular secretory PLA_2. The PLA_2 inhibitor may be substantially homogenous or may be in a partially-purified form by, for example, fractionation using anion exchange chromatography or a dialysed form by, for example, cation exchange chromatography. The inventors have further provided sequencably pure *N. scutatus* and *N. ater* PLA_2 inhibitors.

A PLA_2 inhibitor is a molecule which reduces the activity of a phospholipase enzyme compared to the activity of the phospholipase enzyme in the absence of the inhibitor. The preferred PLA_2 inhibitor is a peptide, polypeptide or protein.

Accordingly, a PLA_2 inhibitor is a substance, such as a peptide, polypeptide and protein, which is capable of inhibiting phospholipase enzyme activity. The inhibitor may also be a polypeptide aggregate such as dimer or other multimer of a polypeptide, fusion polypeptide, peptide fragment or a homologue, analogue or derivative thereof which is capable of inhibiting the catalytic activity of a phospholipase enzyme, in particular a PLA_2 enzyme and more preferably more than one type of PLA_2 enzyme.

Reference herein to a "PLA_2 inhibitor" includes reference to any peptide fragments or parts derived from a polypeptide, polypeptide aggregate or fusion polypeptide or homologue, analogue or derivative thereof, which, although they may have no inhibitory activity may nevertheless be useful in modulating a PLA_2 inhibitor by, for example, competition.

Those skilled in the art will be aware that the amount of phospholipase inhibitor which is required to achieve inhibition may vary, depending upon the phospholipase enzyme being inhibited, the presence of other substances which may interfere with phospholipase activity inhibitor activity, in particular substances derived from the source tissue. Accordingly, the present invention is not to be limited by the quantity or amount of phospholipase inhibitor required to achieve a particular degree of inhibition of enzyme activity.

In a preferred embodiment of the invention, the PLA_2 protein inhibitor described herein is

capable of inhibiting at least 20%, more preferably at least about 50-70% and even more preferably at least about 80% of the PLA_2 activity present in a biological sample such as secretory PLA_2 in serum or tissue fluid.

In particular, the phospholipase inhibitor of the present invention exemplified herein [*N.scutatus* PLA_2 inhibitor (NSI) and *N. ater* PLA_2 inhibitor (NAI)] have been shown by the inventors to inhibit all groups of PLA_2 enzymes against which it has been tested. The molar ratio of NSI:PLA_2 and NAI:PLA_2 are each believed to be about 1:1. It has an IC_{50} value of about 1.5μM for recombinant human non-pancreatic type-II PLA_2. Additionally, NSI and NAI form a stable complex with notexin (a purified PLA_2 enzyme) as judged by elution from a size exclusion column and also prevents radioiodinated notexin from binding to isolated rat brain synaptosomes.

In one embodiment of the invention, the PLA_2 inhibitor is derived from the serum of an animal such as a snake or other reptile, which produces a venom having toxic PLA_2 activity in humans or other animals.

Hereinafter, the term "derived from" shall be taken to refer to the origin of an integer or group of integers from a specified source, but not to the exclusion of other possible source or sources of said integer or group of integers.

In a particularly preferred embodiment of the invention, the PLA_2 inhibitor is derived from a snake.

In a most particularly preferred embodiment, the present invention provides an isolated PLA_2 inhibitory protein derived from *Notechis scutatus* (NSI) or *Notechis ater* (NAI) which is capable of inhibiting more than one type of PLA_2 or is a functionally equivalent, homologue, analogue or derivative thereof of said inhibitor.

The present invention extends to all isoforms of NSI and NAI.

The present invention extends further to a PLA_2 inhibitor molecule wherein said molecule is capable of binding to the active site of the PLA_2 enzyme.

In a particularly preferred embodiment, the PLA_2 inhibitor molecules according to this embodiment are capable of forming an interactive site with a phospholipase enzyme to inhibit the activity of the enzyme.

As used herein, the term "interactive site" shall be taken to refer to the primary, secondary or tertiary structure of a phospholipase inhibitor of the present invention which is in physical relation with a phospholipase enzyme wherein said physical relation is required for the inhibitory activity of said inhibitor, or at least contributed to the inhibitory activity of said inhibitor.

In a more preferred embodiment, a molecule which is capable of forming an interactive site with a phospholipase enzyme mimics the 3-dimensional structure (i.e. tertiary structure) of the *N. scutatus* PLA_2 inhibitor (NSI) or *N. ater* PLA_2 inhibitor (NAI) and, as a consequence, is capable of reproducing the NSI:PLA_2 or NAI:PLA_2 inhibitory interaction.

In this regard, whilst not being bound by any theory or mode of action, the mechanism of interaction between NSI or NAI and the PLA_2 enzyme at least appears to be unique compared to the mode of interaction of other PLA_2 inhibitors with the specific enzymes which they inhibit, thereby accounting for the generality of NSI or NAI inhibitory activity. Those skilled in the art will be aware that once the structure of the interactive site between NSI or NAI and a PLA_2 enzyme is established by standard X-ray crystallographic procedures, it is possible to synthesize peptides or other molecules (mimotypes) which are capable of reproducing the inhibitory function of NSI or NAI. Such mimotypes, whilst capable of forming an interactive site with a phospholipase enzyme may not comprise the same amino acid sequence (i.e. primary structure) as the NSI or NAI α-chain and/or β-chain polypeptide(s). Furthermore, those skilled in the art will be aware that mimotypes may also comprise synthetic molecules such as chemical compounds or anti-idiotypic antibodies of the phospholipase inhibitor of the invention capable of forming an interactive site with a phospholipase. Those skilled in the art will also be aware that

mimotypes may be presented on a carrier molecule or embedded therein, such that the mimotype moiety is presented in a functional conformation capable of inhibiting phospholipase enzyme activity. Accordingly, the present invention clearly extends to any molecule or composition of matter which at least comprises a mimotype of NSI or NAI or the interactive site thereof.

Carrier molecules for presenting a mimotype may comprise amino acid sequences presented as an in-frame fusion polypeptide with a polypeptide mimotype or alternatively, associated with a polypeptide mimotype by means of a disulfide bridge or other covalent bond formation, van der Waals interaction or ionic interaction, amongst others. Alternatively, wherein the mimotype moiety is a chemical compound, the mimotype may be embedded into a polypeptide carrier by any means known to those skilled in the art.

Carrier molecules for presenting a mimotype may also comprise polysaccharide molecules, nucleic acid molecules such as RNA or DNA, biologically inert carriers such as tungsten or gold, amongst others, polymers such as starches, dextrans, glycogen, Percoll (Trademark of Pharmacia Fine Chemicals) or Ficoll (Trademark of Pharmacia Fine Chemicals), amongst others, agarose, polyacrylamide or other couriers known to those in the pharmaceutical and/or biomolecular engineering industries.

Another aspect of the present invention provides an isolated phospholipase inhibitory protein which at least comprises an amino acid sequence which is at least about 40% identical to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or is a homologue, analogue or derivative thereof. The amino acid sequences set forth in SEQ ID NOS: 4-11 relate to tryptic peptides of the *N. scutatus* PLA_2 inhibitory protein β-chain. The amino acid sequence set forth in SEQ ID NO: 1 relates to the derived amino acid sequence of the *N. scutatus* PLA_2 inhibitory protein α-chain. The amino acid sequence set forth in SEQ ID NO: 1 comprises the complete NSI α-chain polypeptide, including a 19 amino acid N-terminal leader peptide which is absent from the N-terminus of the mature protein. The amino acid sequence set forth in SEQ ID NO: 2 relates to the derived amino acid sequence

of the *Oxyuranus scutellatus* PLA_2 inhibitory protein α-chain. The amino acid sequence set forth in SEQ ID NO: 3 relates to the derived amino acid sequence of the *Oxyuranus microlepidotus* PLA_2 inhibitory protein α-chain.

Preferably, the percentage identity is at least about 50%, more preferably at least about 60% and even more preferably at least about 75% identical to the NSI α-chain polypeptide set forth in SEQ ID NO: 1 or the *Oxyuranus spp.* polypeptides set forth in SEQ ID Nos: 2 or 3, still more preferably, the percentage identity is at least about 85%, and even more preferably at least about 95% identical to SEQ ID NO: 1 or 2 or 3.

The percentage identity to the β-chain polypeptide is preferably at least about 40% identical to any one of SEQ ID NOS: 4 to 11 and more preferably at least about 50%, even more preferably at least about 80% and still more preferably at least about 95% identical thereto.

Yet another aspect of the present invention provides an isolated phospholipase inhibitory protein which comprises the amino acid sequence substantially as set forth in any one or more of SEQ ID Nos 12 to 33 or a sequence having at least 40% identity thereto or an amino acid sequence encoded by a nucleotide sequence substantially as set forth in one or more of SEQ ID Nos 34 to 37 or a nucleotide sequence having at least 40% identity thereto or capable of hybridizing to any one of SEQ ID Nos 34 to 37 under low stringency conditions at 42°C. The amino acid and nucleotide sequences set forth in SEQ ID NOs: 12-45 are summarized in Table 1.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or

high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41$ (G+C)% [19]. However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (20).

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch (19). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au..

The present invention clearly extends to the use of the full-length amino acid sequences of both the precursor and mature α-chain and β-chain of the *N. scutatus* PLA_2 inhibitor or *N. ater* PLA_2 inhibitor and high molecular weight and to heteropolymers and recombinant and isolated forms thereof, including fusion polypeptides.

In the present context, "homologues" of a phospholipase inhibitory protein or PLA_2 inhibitory

protein refer to those polypeptides, enzymes or proteins which have a similar inhibitory activity to the NSI or NAI and are at least about 40% identical thereto, notwithstanding any amino acid substitutions, additions or deletions. Homologues may comprise fusion polypeptides between α-chain and β-chain polypeptides with or without additional "spacer" sequences there between to facilitate folding and the ability of said fusion polypeptide to form an interactive site with a phospholipase enzyme. A homologue may be isolated or derived from the same species as the particular PLA_2 inhibitory protein exemplified herein (e.g. *N. scutatus or N. ater*) or alternatively, from a different species or a mixture of same.

Furthermore, the amino acids of a homologous polypeptide may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment, charge or antigenicity, and so on.

"Analogues" encompass PLA_2 inhibitors and polypeptides which are at least about 40% identical to the NSI or NAI or the interactive site thereof, notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein. "Analogues" also encompass polypeptide mimotypes of the phospholipase inhibitor herein described.

The term "derivative" in relation to a PLA_2 inhibitor shall be taken to refer hereinafter to mutants, parts or fragments derived from the functional NSI or NAI or homologues or derivatives thereof which may or may not possess the inhibitory activity of the functional protein. Derivatives include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of a PLA_2 inhibitory protein which comprise fragments or parts of an amino acid sequence disclosed herein are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for

derivatizing peptides are well-known in the art.

Particularly preferred analogues and derivatives of the NSI or NAI polypeptides exemplified herein comprise an amino acid sequence which is capable of binding to the active site of a phospholipase enzyme and/or capable of forming an interactive site with a phospholipase enzyme.

Substitutions which may be included in a homologue, analogue or derivative of any one of SEQ ID NOS: 1 to 3 and/or 4 to 11 and/or 12 to 33 or a phospholipase inhibitor polypeptide comprising amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a phospholipase inhibitory protein is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a phospholipase inhibitory protein is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Naturally-occurring amino acids include those listed in Table 2A. Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2B.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino-or carboxyl-terminal fusions and of the order of 1-4 amino acid residues.

Preferably, the phospholipase inhibitory protein of the invention or a homologue thereof comprises polypeptide chains having an estimated molecular weight of about 25 kDa or 30 kDa as determined by SDS/PAGE or alternatively, about 22-23 kDa or 19-20 kDa as determined by mass spectrometry or alternatively, a fusion polypeptide comprising said polypeptide chains.

Wherein the phospholipase inhibitory protein is a multimeric protein, such as a heteropolymer of α-chain and β-chain polypeptides, it is also preferred that it exist as a trimeric protein having a molecular weight in the range of about 76 kDa to about 120 kDa, more preferably about 84 kDa to about 110 kDa.

In a particularly preferred embodiment of the invention, the phospholipase inhibitory protein or a homologue or analogue thereof is a heterotrimeric α_2:β_1 protein having an estimated molecular weight of about 110 kDa.

The present invention clearly extends to fusion polypeptides comprising one or more α-chain and β-chain polypeptides and mimotypes thereof.

The range provided herein for the estimated molecular weight of a PLA_2 inhibitory protein is merely an approximation and some variation in this estimate may occur. Those skilled in the art will be aware that some variation in the estimated molecular weight of a polypeptide may occur, depending upon the conditions employed to determine said molecular weight.

TABLE 2A

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

TABLE 2B

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle

	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
	D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
	D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
5	D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
	D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
	D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
	D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
10	D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
	D-α-methylleucine	Dmleu	α-napthylalanine	Anap
	D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
	D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
15	D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
20	D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
25	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe
30	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)	

		glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))	
		glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)	
		glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo	
		phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)	
		glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys

L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomo phenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Reference to chemical analogues also includes reference to chemically synthesised molecules or molecules identified following screening of chemical libraries as well as molecules detected following, for example, natural product screening. Useful sources for screening for natural products include coral reefs and sea beds, plants, microorganisms and aquatic and antarctic environments.

The PLA_2 inhibitor or homologue, analogue or derivative thereof herein described is useful in the prophylaxis and treatment of cancer.

Although not intending to limit the present invention to any one theory or mode of action, it is proposed that phospholipase inhibitors alter the regulatory pathway associated with prostaglandin production. After analysing the literature, the inventors summarized diagrammatically the regulation of prostaglandin synthesis. This is shown in Figure 1. This figure shows the interaction between extracellular, membrane associated and cytosolic factors in the production of prostaglandin.

Importantly, Figure 1 shows that secretory PLA_2 ($sPLA_2$) is capable of down-regulating

expression or otherwise reducing the activity of the cycloxygenase, COX2.

Although not wishing to limit the present invention to any one theory or mode of action, it is proposed herein that secretory PLA_2 has a regulatory effect on a cycloxygenase and in particular COX2.

In accordance with the present invention, it is proposed that the administration of a PLA_2 inhibitor such as NSI or NAI or an aforementioned equivalent, derivative or homologue thereof inhibits secretory PLA_2 which thereby reduces expression of COX2. This in turn reduces the catalytic conversion of arachidonic acid to prostaglandin.

Accordingly, another aspect of the present invention contemplates a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor having an amino acid sequence substantially as set forth in any one or more of SEQ ID NOs: 1 to 11 or an amino acid sequence having at least 60% identity to any one or more of SEQ ID NOs: 1 to 11 or a functional derivative or homologue thereof which PLA_2 inhibitor or derivative or homologue reduces the level or activity of secretory PLA_2.

In a particular embodiment the present invention contemplates a method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor having an amino acid sequence substantially as set forth in any one or more of SEQ ID NOs: 1 to 11 or 12 to 33 or an amino acid sequence having at least 60% identity to any one or more of SEQ ID NOs: 1 to 11 or 12 to 33 or a functional derivative or homologue thereof which PLA_2 inhibitor or derivative or homologue reduces the level or activity of secretory PLA_2 thereby reducing expression of a genetic sequence encoding a cycloxygenase or reducing cycloxygenase activity.

Yet another aspect of the present invention provides a biological composition useful for the

treatment and/or prophylaxis of cancer in a target animal or bird such as a human, primate, livestock animal or companion animal said composition comprising a PLA_2 inhibitor such as but not limited to the PLA_2 defined by any one of amino acids sequences set forth in SEQ ID NOs: 1-11 or 12 to 33 or a derivative, homologue, analogue or functional equivalent thereof.

The biological composition according to this aspect of the present invention may also contain other active molecules such as anti-cancer agents, immune-potentiating molecules and/or pharmaceutical compounds which diminish any side-effects of the PLA_2 inhibitors or other active molecules.

The active molecule(s) of the biological composition is/are contemplated to exhibit PLA_2 inhibitory activity and consequently anti-cancer activity in animals and birds when administered by any means including by intravenous, intraperetoneal, sub-cutaneous, topical or oral administration. Variations in dosage administration occur depending, for example, on the activity of the phospholipase enzyme required to be inhibited and the IC50 of the inhibitor, the intended purpose of administration, such as whether for use as an anti-inflammatory agent or as an anti-toxin and particularly in the case of toxic poisoning and the delay between the onset of symptoms and the commencement of treatment. Dosage regimen may be adjusted without undue experimentation by those skilled in the art to provide the optimum therapeutic response. For example, several divided doses may be administered in one or more of daily, hourly, weekly or monthly or in other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation.

The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent, preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Tablets or powders or granules may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Additionally, sweeteners or dietary formulae may be included to improve their palatability to a specific animal subject. Optionally, such solid compositions be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

The active compounds may also be administered in dispersions prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

Biological compositions suitable for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating

action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about, for example, by the use in the compositions of agents delaying absorption.

Sterile injectable solutions are prepared by incorporating the active molecules in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active molecule(s) into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The biological compositions of the present invention may also be delivered by a live delivery system such as using a bacterial expression system to express the PLA_2 inhibitory protein in bacteria which can be incorporated into gut flora. Alternatively, a viral expression system can be employed. In this regard, one form of viral expression is the administration of a live vector generally by spray, feed or water where an infecting effective amount of the live vector (e.g. virus or bacterium) is provided to the animal. Another form of viral expression system is a non-replicating virus vector which is capable of infecting a cell but not replicating therein. The non-replicating viral vector provides a means of introducing to the human or animal subject genetic material for transient expression therein to produce the

PLA_2 inhibitory protein. The mode of administering such a vector is the same as a live viral vector.

The carriers, excipients and/or diluents utilised in the biological compositions of the present invention should be acceptable for human or veterinary applications. Such carriers, excipients and/or diluents are well-known to those skilled in the art. Carriers and/or diluents suitable for veterinary use include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. Except insofar as any conventional media or agent are incompatible with the active ingredient, use thereof in the composition is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The compositions of this invention may include other agents conventional in the art. For example, compositions suitable for oral administration may include such further agents as dietary formulae, binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

The present invention further provides an agent for use in treating or preventing cancer, said agent comprising a PLA_2 inhibitor or a functional derivative, homologue or analogue thereof.

Still another aspect of the present invention contemplates the use of a PLA_2 inhibitor or functional derivative, homologue or analogue in the manufacture of a medicament for the

treatment or prophylaxis of cancer in an animal (e.g. human or bird).

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Purification of Phospholipase A_2 Inhibitor from Snake Blood

Tiger snake (*N. scutatus*) and Tasmanian tiger snake *(N. ater)* blood were collected and allowed to clot. The blood is then centrifuged at 1,500 x g for 15 minutes. The serum is then collected and stored at -20°C. Serum was extensively dialysed against 0.01M ammonium acetate (NH_4OAc), pH 7.0. The *N.scutatus* phospholipase A_2 inhibitor (NSI) and *N. ater* (NAI) were purified using anion exchange chromatography.

Dialysed serum was loaded (up to 15mL at ~20mg/mL) onto a DEAE-Sephacel column (20 x 1.5cm) that has been equilibrated with 0.01M NH_4OAc, pH 7.0 at a flow rate of 0.5mL/min. A step gradient was then developed as follows; 0.1 NH_4OAc, 0.25M NH_4OAc, 0.5 NH_4OAc and 1.0M NH_4OAc (all pH 7.0). The eluent was monitored at 280nm with an Isco type 11 detector. The concentration of NH_4OAc was not increased until the preceding peak has fully eluted. NSI and NAI were eluted the 0.5M NH_4OAc step. The procedure was performed at 4°C.

The sample was then concentrated by lyophilisation and then resuspended in water and stored at -20°C. Alternatively, if a large volume was collected (>15mL), the sample was concentrated using an Amicon ultrafiltration device fitted with a YM 10 membrane. This semi-purified preparation (SPP) of NSI or NAI was approximately 90-95% pure.

NSI and NAI can be purified to >98% purity using cation exchange chromatography. A Mono-S HR 5/5 column was equilibrated with 10mM sodium acetate pH 5.5. The SPP NSI or NAI fraction was applied and a gradient developed with 430mM sodium acetate pH 5.5 as follows:

(i) 0-3 minutes 0%;

(ii) 3-8 minutes 0-20%;

(iii) 8-20 minutes 20-40%;

(iv) 20-25 minutes 40-60%; and

(v) 25-30 minutes 60-100%.

NSI and NAI eluted in the 20-40% section of the gradient. (Figure 1a and 1b).

The amino acid sequence for NAI are shown in SEQ ID NOS 12 to 33. Corresponding nucleotide sequences are shown in SEQ ID NOS 34 to 37. The amino acid sequence of the leader system and corresponding nucleotide sequence are shown in SEQ ID NOS 38 to 45.

EXAMPLE 2

Phospholipase A_2 assays and inhibition of Phospholipase A_2 activity by NSI

Phospholipase A_2 activity was assigned using a modification of the method of Radvanyi *et al.* (17). This assay is based on the ability to measure the fluorescence emitted by an artificial substrate after it has been cleaved by a PLA_2 enzyme. The level of fluorescence is proportional to the amount of cleaved substrate which is in turn proportional to enzymatic activity. The phospholipid substrate, labelled in the sn-2 position with 10-pyrenyldecanoic acid, forms micelles upon addition to the reaction medium. The fluorescence of the substrate is quenched by pyrene-pyrene interactions. Upon hydrolysis the free 10-pyrenyldecanoic acids are absorbed by bovine serum albumin (BSA) and the fluorescence emitted is measured. The artificial substrate 1-hexadecanoyl-2-(1-predecanoyl)-sn-glycero-3-phosphocholine (10pPC [Molecular Probes, Inc.]) was dissolved (1mg) in 5.87mL 95% v/v ethanol to yield a 0.2M stock solution. 200μL aliquots were stored at -20 C for up to 3 months.

To 1mL of assay buffer (50mM Tris [hydroxymethyl]methylamine-HCl[Tris]), pH7.5, 100mM NaCl, and 1mM ethylenediaminetetra-acetic acid [EDTA]) the following were added sequentially; 16μL of a 1:0.6 v/v mixture of 10% w/v BSA and 1M $CaCl_2$ (0.1% and 2μM final concentration respectively), 10μL 10pPC stock solution, injected quickly to facilitate micellular formation. To this, 35μL of a test sample, PLA_2 source plus SPP or water, or saline/BSA, was added. This solution was mixed well with shaking.

The substrate was excited at 345nm and the fluorescent spectrometer for 4 minutes.

EXAMPLE 3

Inhibition of non-snake venom phospholipase A_2 enzymes by *N.scutatus* phospholipase A_2 inhibitor

Phospholipase A_2 enzyme activity assays were performed as described in Example 2. The assay was performed as above except that 10pPG (1-hexadecanoyl-2-(1-predecanoyl)-sn-glycero-3-phosphoglycerol, ammonium salt) was used as the substrate, because most of the non-snake venom PLA_2s are not active on 10pPC. Also, saline, rather than water was used for the negative control.

PLA_2 enzymes were diluted to achieve an enzyme activity sufficient to produce a change of 250 fluorescent units over 70-80 seconds in the enzyme assay, in the absence of inhibitor. Samples tested were; *N. scutatus* venom (positive control), bee venom phospholipase A_2 (*Apis meliffera*), porcine pancreatic phospholipase A_2 PLA_2 (*Sus scrofa*), and osteo-arthritis synovial fluid aspirates and rheumatoid arthritis-synovial fluid aspirates. Dilutions of phospholipase A_2-containing samples which were used were as follows; *N.scutatus* venom 1/30, bee venom phospholipase A_2 1/400, porcine pancreatic phospholipase A_2 1/3, all 1mg/ml. Osteo-arthritis, undiluted to 1/10 and rheumatoid-arthritis-synovial, 1/30, 25-36mg/mL total protein. It should be noted that not all of the OA or RA samples meet with the activity criteria of 250 fluorescent intensity units over 70-80 seconds, however, the activity was consistent and measurable.

Dilutions of the SPP varied according to the phospholipase A_2 tested. Two dilution groups were used for a 7.13mg/mL solution of the SPP:

Group 1; 1/14, 1/50, 1/330 and 1/660. Phospholipase A_2 sources challenged with this group were *N.scutatus* venom, porcine pancreatic phospholipase A_2 and bee venom phospholipase A_2.

Group 2; ½, 1/7, 1/14 and 1/50. Phospholipase A_2 sources challenged with this group were, all OA and RA samples.

As shown in Figure 13A, the SPP fraction of *N.scutatus* phospholipase A_2 inhibitor strongly inhibited bee venom phospholipase A_2 at all concentrates tested. A 50% inhibition of porcine pancreas phospholipase A_2 was observed at a 1/4 dilution of SPP.

As shown in Figure 13B, the SPP fraction of *N.scutatus* phospholipase A_2 inhibitor significantly inhibited the three osteoarthritis samples tested, with about 40-60% inhibition of enzyme activity being observed at a ½ dilution of SPP. In two of the three samples tested, about 50% inhibition of phospholipase A_2 activity was observed at the 1/7 dilution level of SPP. Weak, albeit detectable inhibition of phospholipase A_2 in the rheumatoid arthritis sample tested was also detected at the ½ dilution of SPP.

These data indicate that the *N. scutatus* venom phospholipase A_2 inhibitor is a broad-spectrum inhibitor of non-snake venom-derived phospholipase A_2 activities.

EXAMPLE 4

Inhibition of a variety of snake venom phospholipase A_2 activities by partially-purified *N. scutatus* phospholipase A_2 inhibitor

Using the SPP fraction prepared according to Example 1, inhibition of the phospholipase A_2 activities of a wide range of snake venoms was tested. The venoms tested were; *N.scutatus* (homologous venom), *P.textilis*, *N.melanoleuca* (family; Elapidae), *V.russelli* (family; Viperidae), *A. bilineatus*, *B.alternatus* and *C.atrox* (family; Viperidae, subfamily; Crotalinae.

First, an appropriate dilution of venom was established for use in the assay described in Example 2. The criteria required a substantial change in fluorescent intensity over a relatively short period of time. Venoms were diluted to achieve a phospholipase A_2

enzyme activity sufficient to produce a change of 250 fluorescent intensity units over 70-80 seconds in the absence of any inhibitor. As such all venoms showed similar PLA_2 activity in the assay. A 1mg/mL solution of each venom was made up fresh when it was to be tested. Dilutions (of the 1mg/mL solution) used in the assay are as follows; *N.scutatus* 1/200, *P.textilis* 1/20, *N.melanoleuca* 1/150, *V.russelli* 1/15, *A.bilineatus* 1/20, *B.alternatus* 1/10 and *C.atrox* 1/10.

The SPP fraction was also diluted prior to testing against each venom. The dilutions were; 1/2, 1/8, 1/12, 1/50, 1/100 and 1/200 of a 1.11mg/mL solution.

The SPP dilutions were incubated with each diluted venom sample in the ratio 2.5:1 (v/v) before assaying phospholipase A_2 enzyme activity. Three assays were performed for each dilution of SPP on each day. Control samples were assayed both before and after each dilution was tested. The control consisted of venom plus water in the same ratio as the SPP:venom. Three batches were assayed daily with separate controls for each batch. All samples were prepared at the same time and then selected randomly for testing. All samples being tested were kept on ice. Samples not used immediately were stored at -20°C.

Results were determined as percentage inhibition compared to control values (Figures 14A and 14B). The SPP fraction of *N.scutatus* phospholipase A_2 inhibitor was most effective at inhibiting the activities of *N.scutatus* snake venom phospholipase A_2, with at least 80% inhibition of the related *N.melanoleuca* phospholipase A_2 being observed at all dilutions of SPP tested. Significant inhibition of phospholipase A_2 activities derived from the more distantly related species were also observed at high concentrations of the SPP fraction, wherein 50% inhibition or *V.russelli* phospholipase A_2 was observed at a 1/25 dilution of SPP and a 50% inhibition of the *A.bilineatus* and *B.alternatus* phospholipase A_2 activities was observed at about a 1/12 dilution of SPP and a 50% inhibition of the *P.textilis* and *C.atrox* phospholipase A_2 activities was observed at about a 1/2-1/8 dilution of SPP.

These data indicate that the *N.scutatus* venom phospholipase A_2 inhibitor is a broad-spectrum
inhibitor of snake venom phospholipase A_2 enzymes.

EXAMPLE 5

Mixed micelle assay of recombinant human type II phospholipase A_2 and inhibition of enzyme activity using *N. scutatus* phospholipase A_2 inhibitor

An alternative assay of phospholipase A_2 activity was a mixed micelle phosphatidylethanolamine (PE/sodium deoxycholate (DOC) assay modified from a method of Seilhamer *et al* (18). This assay is particularly suited to quantifying recombinant human phospholipase A_2 activity as it utilises a PE/DOC substrate. The PE substrate was prepared by dissolving freshly desiccated [^{14}C]PE (Amersham) in 2% DOC, then diluting this to 0.22μmoles PE and 0.04% DOC per sample in assay buffer (50mM Tris-HCl, pH 8.5, 2mM $CaCl_2$, 150mM NaCl, 0.04% DOC). The sample was prepared by mixing 10μL of the test material with 10μL 10mM Tris-HCl, pH 7.4 and incubating for 10 minutes at 37°C. The reaction was started by the addition of 25μL pre-warmed substrate and terminated by the addition of 10μL 100mM EDTA. The reaction mixture (30μL) was spotted and dried onto silica TLC plates. The plates were chromatographed using chloroform:methanol:acetic acid (90:10:1) as solvent. The dried plates were then exposed overnight with Kodak X-OMAT AR film. Radioactivity at the origin was counted and the percent hydrolysis by phospholipase A_2 determined.

As shown in Figure 15, the recombinant human phospholipase A_2 activities is significantly inhibited at 0.1-1.0μM concentrations of *N.scutatus* phospholipase A_2 inhibitor. The IC_{50} of *N. scutatus* phospholipase A_2 inhibitor for recombinant human non-pancreatic phospholipase A_2 is approximately 1.5μM.

EXAMPLE 6

pH Optimum and temperature stability of *N.scutatus* venom phospholipase A_2 inhibitor

The pH stability was investigated by altering the pH of the solution in which the SPP (0.4mg/mL) was dissolved and then testing this in the phospholipase A_2 assay. The assay was performed as described in Example 2, using *N.scutatus* venom as the phospholipase A_2 source (1/200 dilution of a 1mg/mL with 10pPC as substrate). All samples were performed in triplicate with appropriate positive and negative controls. The pH values tested were: 2, 4, 6, 7, 8, 9, 10 and 12.

The temperature stability was assessed in the same manner as the pH stability. Samples were heated, or cooled, at the appropriate temperature and then immediately tested in the phospholipase A_2 assay. Temperatures examined were; 4°C, 25°C, 37°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C.

For both experiments samples were not preincubated with the venom as the stability of the phospholipase A_2 under the varying pH and temperature values could not be assured. However, the ratios phospholipase A_2 to inhibitor used in the preceding Examples were maintained in this procedure.

NSI was stable in the pH range 4.0-12.0, with activity declining at extreme acidic pH values. NSI was also stable at the temperatures tested. Thus, NSI is a highly-stable protein.

EXAMPLE 7

Activity of the *N.scutatus* phospholipase A_2 inhibitor following de-glycosylation of the α-chain

The α-chain was deglycosylated with N-glycosidase F (cleaves N-linked sugars) or O-

glycosidase (cleaves O-linked sugars) as follows: 10μg (10μL) of the SPP was denatured with an equal volume of 1% (w/v) SDS followed by boiling for 2 minutes. To this 90μL 20mM sodium phosphate buffer, pH 7.2, 50mM EDTA, nonidet P-40, 0.5% v/v was added followed by a further 2 minutes boiling. The SPP was then incubated with 0.4U N-glycosidase or 2.5mU O-glycosidase for 16 hours at 37°C. A sample was then run on SDS-PAGE under reducing conditions. The gel was then blotted onto nitrocellulose and sugar residues detected with the Boehringer Mannheim DIG glycan detection kit as per manufacturers instructions. Appropriate controls were performed. A duplicate gel was run and silver stained to determine the shift in molecular weight of the α-chain following deglycosylation.

It was determined that only N-linked sugars were present on the α-chain. As such, the α-chain was deglycosylated with N-glycosidase F as outlined above except that SDS and nonidet P-40 were omitted as were the boiling steps. This was to ensure that NSI was not irreversibly denatured by boiling or SDS treatment. Deglycosylation was confirmed with the DIG glycan detection kit and the shift in molecular weight following SDS-PAGE. The sample was then assayed for inhibitory activity on *N.scutatus* venom (1/300 dilution of 1mg/mL solution dissolved in saline/0.1% w/v BSA). Native NSI was used as the positive control.

The formation of the NSI intact complex following deglycosylation of the α-chain was determined using size exclusion chromatography. The deglycosylated SPP (containing NSI) was run on a Superdex 75 column (3.2mm x 30mm) using the Pharmacia SMART HPLC system in 0.1M NH_4OAc pH 7.0. The column was calibrated with molecular weight standards. Native SPP was run as a positive control.

The de-glycosylated NSI retained activity compared to the native inhibitor, consistent with observations in respect of both *A.bilineatus* and bee venom phospholipase A_2 inhibitors.

However, the de-glycosylated NSI exhibited a different elution profile from Superdex 75

compared to the native inhibitor, with significantly higher molecular weight species being present, possible due to the formation of functional high molecular weight aggregates involving the de-glycosylated α-chain. Additionally, the size of the assembled NSI complex differed slightly from native NSI due to the altered glycosylation status of the assembled complex.

EXAMPLE 8

Determination of the *N.scutatus* phospholipase A_2 inhibitor complex formation with notexin

The native molecular weight of NSI was determined using size exclusion chromatography using a Pharmacia Superose 12 HR10/30 column attached to a Waters 600 series HPLC system. Elution buffer was 0.1M NH_4OAc, pH 7.0 at a flow rate of 0.5mL/min. NSI (60μg) was loaded on the column. The column was calibrated with molecular weight standards. The formulation of a stable complex between NSI and notexin was also investigated using size exclusion chromatography. The SPP (150μg) and notexin (100μg) were incubated for 30 minutes followed by elution on the Superose column. The NSI and notexin mixture eluted from Superose 12 immediately before NSI, confirming the ability of NSI to bind to notexin.

The peaks were collected and components identified by SDS-PAGE followed by silver staining to confirm their identities.

EXAMPLE 9

Effects of NSI on Cancer Cells *in vivo*

Nude mice experiments were conducted to investigate the effects of NSI (a PLA_2 inhibitor), NS398 (a COX2 inhibitor) and a combination of NSI and NS398 on the growth of cancers in the mice. A total of 8 cell lines were employed. The cancers selected were

PC-2 [ATCC No. CRL1435] which is a prostate cancer cell line (adenocarcinoma epithelial cells) and LNCaP [ATCC No. CRL10995/ CRL1740] which is also a prostate cancer (carcinoma epithelial cells). In addition, the following cell lines were also used.

Tca 8113	tongue cancer
Acc-2	adenoid cystic keratin
Acc-3	adenoid cystic carcinoma
BGC-823	stomach carcinoma (62 yrs male) [epithelial like cell]
SGC-7901	stomach adenocarcinoma (56 yrs female) [metastasis to lymph node, epithelial like cell]
SPC-A-1	lung adenocarcinoma

NSI, NS398 or the combination of NSI and NS398 were administered by either subcutaneously or intraperitoneally. After the first injection, administration was 3 times a week for 6 weeks.

Two tumours were induced per animal as follows:

1st phase:	1 x 8 cell lines	8 mice

2nd phase:

Per mode of injection:

NSI	7 mice
NS398	7 mice
NSI/NS398	7 mice
Control	7 mice
	28 mice

(2 modes of injections;
subcutaneous and intraperitonal) 56 mice

Total 64 mice

Number of cells required:

1st phase:

8 tumour cells lines 10^6 cell/line

2nd phase:

2 tumours $28x10^6$ cells/tumour

Dosages of the compounds were as follows:

NSI (MW 110,000): 1.0 μmol/kg body weight

Approximately 2.2 mg of NSI was administered per mouse over 3 injections.

NS398 (MW314): 0.3-5 μg/kg body weight.

Approximately 31.4 μg of NS398 was administered per mouse over 3 injections.

The following time line for the nude mice experiments was observed:

1st Phase:		Week
	Grow cells (8 cell lines) to 10^6 cells	1st-2nd
2nd Phase:		
	Inoculation to induce tumours (8 cell lines)	3rd-6th
	Grow cells (2 cell lines) $28x10^6$ cells	4th-6th
3rd Phase:		
	Animal inoculations and tumour growth	6th-12th
Total:		**12 weeks**

The results are shown graphically in Figures 2 to 7 and in corresponding Tables 3 to 8, respectively.

Referring to the figures and the tables, the cancer cell line tested was BGC823. Day 0 is the day the first dose of inhibitor is administered. The volume of cancer is then determined. As shown in Figure 2 (Table 3), cancer size is reduced in the presence of NSI inhibitor (subcutaneously administered). In Figure 3 (Table 4), the inhibition by NSI is even greater when intraperitionally administered.

Figure 4 (Table 5) and Figure 5 (Table 6) show the effects of NS398 administered by the subcutaneous and interperitioneal routes, respectively. Again, inhibition of the cancer is observed. The combination of NSI and NS398 is shown in Figure 6 (Table 7) and 7 (Table 8).

These data show that inhibiting COX2 does reduce the volume of cancer in nude mice.

EXAMPLE 10

Effects of NSI on Cancers *in vivo*

The methodology of Example 5 was again applied to nude mice using the same protocol.

The results are shown in Figures 8 to 12.

Figure 8 shows the growth of tumours BGC-823 and SGC-7901 in nude mice without inhibitors.

Figures 9 and 10 show the effects of tumour growth in the presence of NSI or NS398 (Figure 9) or the combination of NSI and NS398 (Figure 10).

Similar data are shown in Figures 11 and 12.

Again NSI and NS398 individually inhibit tumour growth but the combination of NSI and NS398 is not demonstrably better than the individual inhibitors.

In summary, the data indicate that NSI is a potent inhibitor of BCG823 abdominal growth and in is more effective than NS398. Synergism between NSI and NS398 has not yet been observed.

EXAMPLE 11

Effects of cytokines on Inhibition of Cancer by NSI

$sPLA_2$ expression is enhanced by cytokines such as IL-1 TNFα. Monoclonal antibodies are commercially available against these cytokines as well as PLA_2 and COX2. Those antibodies are used to monitor $sPLA_2$ expression and/or activity in response to PLA_2 inhibitors and in response to inhibitors of the cytokines. By reducing PLA_2 expression or activity, tumour growth is expected to be greatly reduced.

EXAMPLE 12

Effects of NAI on cancers *in vivo*

Similar results to these described above are obtainable using NAI from *N. ater*.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 3

BGC-823 Control Epidermis NSI Epidermis

Day	control	max	min	inhibitor	max	min	t	P
11	154.76	190.20	119.32	60.17	79.66	40.68	2.34	0.05
14	496.89	615.26	378.52	214.69	323.91	105.47	1.75	>0.1
16	741.78	852.48	631.08	377.68	555.34	200.02	1.74	>0.1
18	1097.65	1260.81	934.49	567.41	835.72	299.10	1.69	>0.1
21	1680.40	2072.09	1288.71	910.51	1320.88	500.14	1.36	>0.1
23	2341.97	2753.51	1930.43	1216.20	1761.93	670.47	1.65	>0.1
25	2786.51	3228.10	2344.92	1623.79	2327.66	919.92	1.40	>0.1

TABLE 4

BGC-823 Control Abdomen NSI Abdomen

Day	control	max	min	inhibitor	max	min	t	P
11	228.10	305.27	150.93	10.24	18.04	2.44	2.81	0.02
14	640.70	852.78	428.62	5.00	8.73	1.27	3.00	0.02
16	920.72	1194.55	646.89	10.39	19.09	1.69	3.32	0.01
18	1430.13	1833.83	1026.43	46.77	85.94	7.60	3.41	0.01
21	2046.44	2608.89	1483.99	119.95	209.96	29.94	3.38	0.01
23	2461.80	3134.96	1788.64	168.25	284.63	51.87	3.36	0.01
25	3136.62	4004.11	2269.13	303.86	508.56	99.16	3.18	0.01

TABLE 5

BGC-823 Control Epidermis NS398 Epidermis

Day	control	max	min	inhibitor	max	min	t	P
11	154.76	190.20	119.32	14.61	25.33	3.89	3.78	0.01
14	496.89	615.26	378.52	49.56	89.87	9.25	3.58	0.01
16	741.78	852.48	631.08	43.70	73.69	13.71	6.09	0.01
18	1097.65	1260.81	934.49	108.62	187.65	29.59	5.46	0.01
21	1680.40	2072.09	1288.71	206.84	341.27	72.41	3.56	0.01
23	2341.97	2753.51	1930.43	325.39	535.64	115.14	4.36	0.01
25	2786.51	3228.10	2344.92	513.48	837.84	189.12	4.15	0.01

15

TABLE 6

BGC-823 Control Abdomen NS398 Abdomen

Day	control	max	min	inhibitor	max	min	t	P
11	228.10	305.27	150.93	18.29	27.67	8.90	2.70	0.02
14	640.70	852.78	428.62	73.22	106.50	39.94	2.64	0.05
16	920.72	1194.55	646.89	146.69	207.98	85.39	2.76	0.05
18	1430.13	1833.83	1026.43	246.95	347.59	146.31	2.84	0.02
21	2046.44	2608.89	1483.99	445.02	615.12	274.91	2.73	0.02
23	2461.80	3134.96	1788.64	632.81	865.08	400.53	2.57	0.05
25	3136.62	4004.11	2269.13	760.08	1024.53	495.62	2.62	0.05

TABLE 7

BGC-823 Control Epidermis NSI+NS398 Epidermis

Day	control	max	min	inhibitor	max	min	t	P
11	154.76	190.20	119.32	19.49	38.97	0.00	3.34	0.02
14	496.89	615.26	378.52	69.37	138.74	0.00	3.12	0.01
16	741.78	852.48	631.08	112.41	218.77	6.05	4.10	0.01
18	1097.65	1260.81	934.49	187.70	344.66	30.75	4.02	0.01
21	1680.40	2072.09	1288.71	511.63	956.80	66.46	1.97	0.10
23	2341.97	2753.51	1930.43	575.60	1068.35	82.86	2.75	0.05
25	2786.51	3228.10	2344.92	745.02	1367.89	122.14	2.67	0.05

15

TABLE 8

BGC-823 Control Abdomen NSI+NS398 Abdomen

Day	control	max	min	inhibitor	max	min	t	P
11	228.10	305.27	150.93	58.66	89.99	27.33	2.03	0.10
14	640.70	852.78	428.62	131.83	215.02	48.63	2.23	0.05
16	920.72	1194.55	646.89	171.00	279.20	62.79	2.55	0.05
18	1430.13	1833.83	1026.43	282.63	449.52	115.74	2.63	0.05
21	2046.44	2608.89	1483.99	689.83	1142.44	237.22	1.88	0.10
23	2461.80	3134.96	1788.64	897.55	1478.49	316.61	1.76	>0.1
25	3136.62	4004.11	2269.13	1104.17	1752.86	455.48	1.88	0.10

BIBLIOGRAPHY

1. World Health Organisation *World Health Statistics Annual* World Health Organisations, 1995.
2. Sheng *et al. J. Clin. Investigation 99:* 2254-2259, 1997.
3. Giovannucci *et al. Ann. Intern. Med. 121:* 241-246, 1994.
4. Giovannucci *et al. N. Engl. J. Med. 333:* 609-614, 1995.
5. Marnelt *et al. Cancer Res. 52:* 5575-5589, 1992.
6. Thun *et al. N. Engl. J. Med. 325:* 1593-1596, 1991.
7. Marnett *Prov. Med. 24:* 103-106, 1995.
8. Meade *et al. J. Biol. Chem. 268:* 6610-6614, 1993.
9. Laneuville *et al. J. Pharmacol. Exo. Ther. 271:* 927-934, 1994.
10. Gierse *et al. Biochem J. 305:* 479-484, 1995.
11. Williams and DuBois *Am. J. Physiol. 270:* G393-G400, 1996.
12. Nathans *et al. Cold Spring Harbor Symp. Quant. Biol. 53:* 893-900, 1988.
13. Herschman *Ann. Rev. Biochem. 60:* 281-319, 1991.
14. Dennis *J. Biol. Chem. 269:* 13057-13060, 1994.
15. Dennis *TIBS 22:* 1-2, 1997.
16. Reddy *et al. J. Biol. Chem. 272:* 13591-13596, 1997.
17. Radvanyi *et al. Anal. Biochem, 177:* 103-109, 1989.
18. Seilhamer *et al. J. Biochem. 106:* 38-42, 1989.
19. Needleman and Wunsch *J. Mol. Biol. 48:* 443-453, 1970.

CLAIMS:

1. A method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a phospholipase inhibitor or a functional derivative or homologue thereof.

2. A method according to claim 1 wherein the phospholipase inhibitor or derivative or homologue reduces the levels and/or activities of a phospholipase to an extent to reduce the growth and/or development of cancer cells.

3. A method according to claim 1 or 2 wherein the growth and/or development of cancer is in an animal.

4. A method according to claim 3 wherein the animal is a human.

5. A method according to claim 1 wherein the phospholipase inhibitor reduces the volume of cancer in the animal or avian species.

6. A method according to claim 1 wherein the phospholipase inhibitor inhibits more than one type of phospholipase type A_2 (PLA_2).

7. A method according to claim 6 wherein the PLA_2 inhibitor is derived from *Notechis scutatus* or *Notechis ater*.

8. A method according to claim 7 wherein the PLA_2 inhibitor comprises an amino acid sequence substantially set forth in SEQ ID NO:1 or any one of SEQ ID NOs:4 to 11 or 12 to 33.

9. A method according to claim 6 wherein the PLA_2 inhibitor comprises an amino

acid sequence substantially set forth in SEQ ID NO:2 or SEQ ID NO:3.

10. A method according to any one of claims 1 to 9 wherein the phospholipase inhibitor inhibits secretory PLA_2 which in turn reduces expression of COX2 thereby reducing catalytic conversion of arachidonic acid to prostaglandin.

11. A biological composition useful for the treatment and/or prophylaxis of cancer in a target animal or bird such as a human, primate, livestock animal or companion animal said composition comprising a PLA_2 inhibitor such as but not limited to the PLA_2 defined by any one of amino acids sequences set forth in SEQ ID NOs: 1 to 11 or 12 to 33 or a derivative, homologue, analogue or functional equivalent thereof.

12. A method for controlling the growth and/or development of a cancer in an animal or avian species said method comprising administering to said animal or avian species an effective amount of a PLA_2 inhibitor having an amino acid sequence substantially as set forth in any one or more of SEQ ID NOs: 1 to 11 or 12 to 33 or an amino acid sequence having at least 60% identity to any one or more of SEQ ID NOs: 1 to 11 or 12 to 33 or a functional derivative or homologue thereof which PLA_2 inhibitor or derivative or homologue reduces the level or activity of secretory PLA_2 thereby reducing expression of a genetic sequence encoding a cycloxygenase or reducing cycloxygenase activity.

13. A biological composition useful for the treatment and/or prophylaxis of cancer in a target animal or bird such as a human, primate, livestock animal or companion animal said composition comprising a PLA_2 inhibitor such as but not limited to the PLA_2 defined by any one of amino acids sequences set forth in SEQ ID NOs: 1 to 11 or 12 to 33 or a derivative, homologue, analogue or functional equivalent thereof.

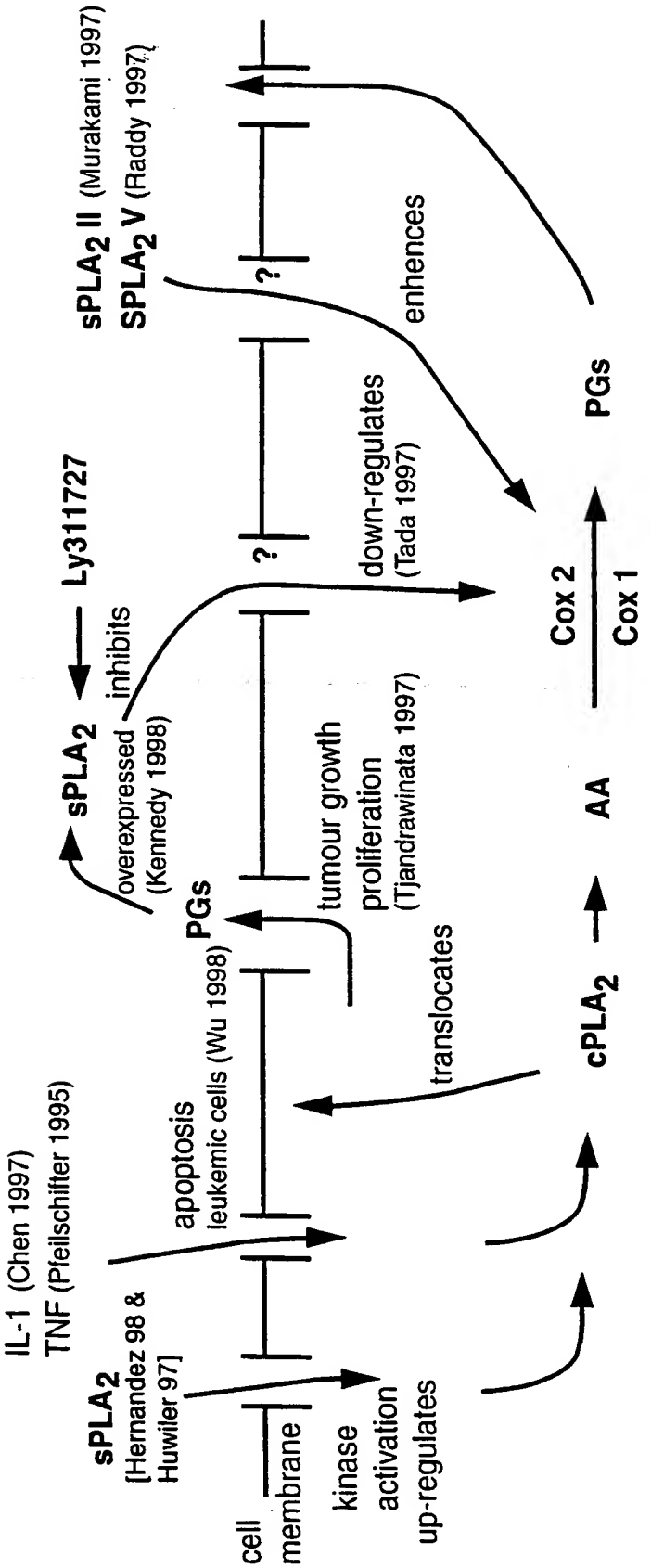
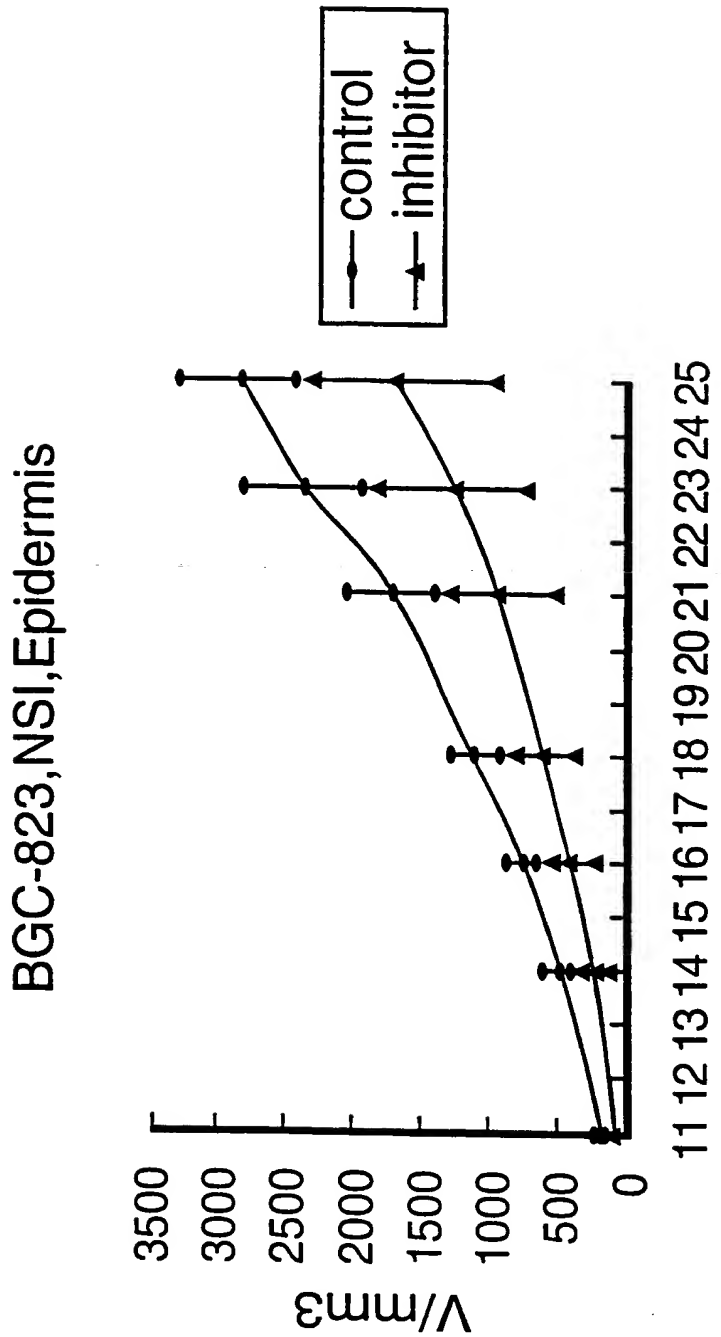

sPLA2 II (Murakami 1997)
SPLA2 V (Raddy 1997)
?
enhences
Ly311727
inhibits
sPLA2
overexpressed (Kennedy 1998)
?
down-regulates (Tada 1997)
PGs
Cox 2
Cox 1
PGs
tumour growth proliferation (Tjandrawinata 1997)
translocates
IL-1 (Chen 1997)
TNF (Pfeilschifter 1995)
apoptosis leukemic cells (Wu 1998)
cPLA2
AA
sPLA2 [Hernandez 98 & Huwiler 97]
kinase activation up-regulates
cell membrane

Figure 1


BGC-823,NSI,Epidermis
control
inhibitor
V/mm3
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3000
2500
2000
1500
1000
500
0
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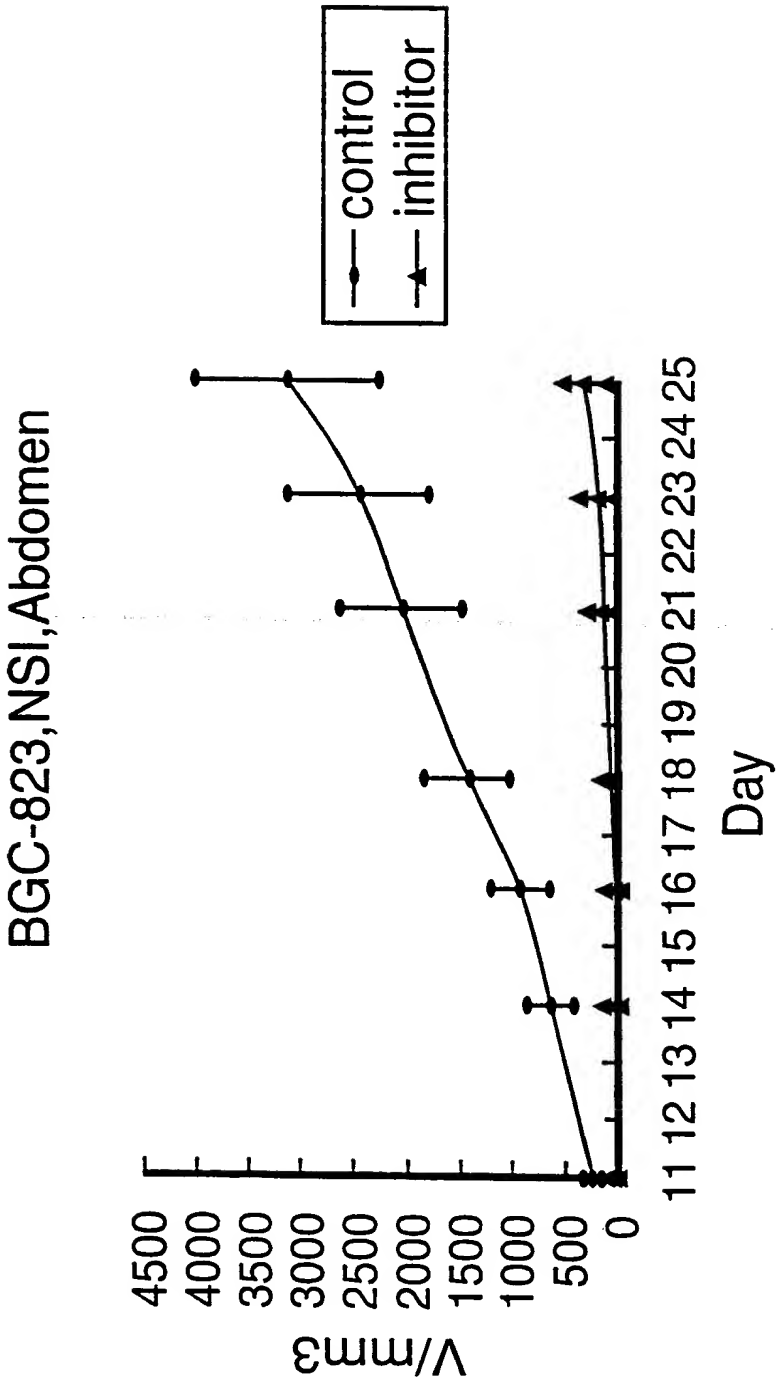
Day

Figure 2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control
inhibitor
V/mm3
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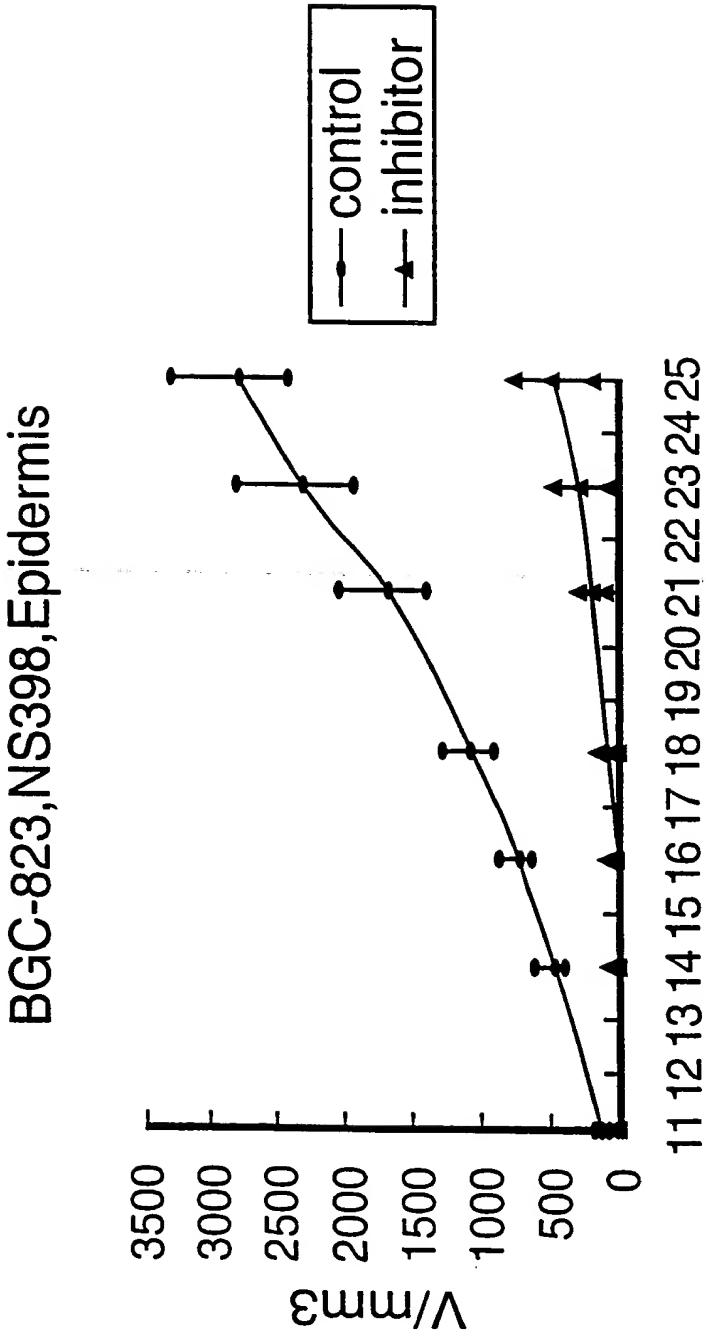
3500
3000
2500
2000
1500
1000
500
0
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Day

Figure 3


BGC-823,NS398,Epidermis
control
inhibitor
V/mm3
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3000
2500
2000
1500
1000
500
0
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Day

Figure 4

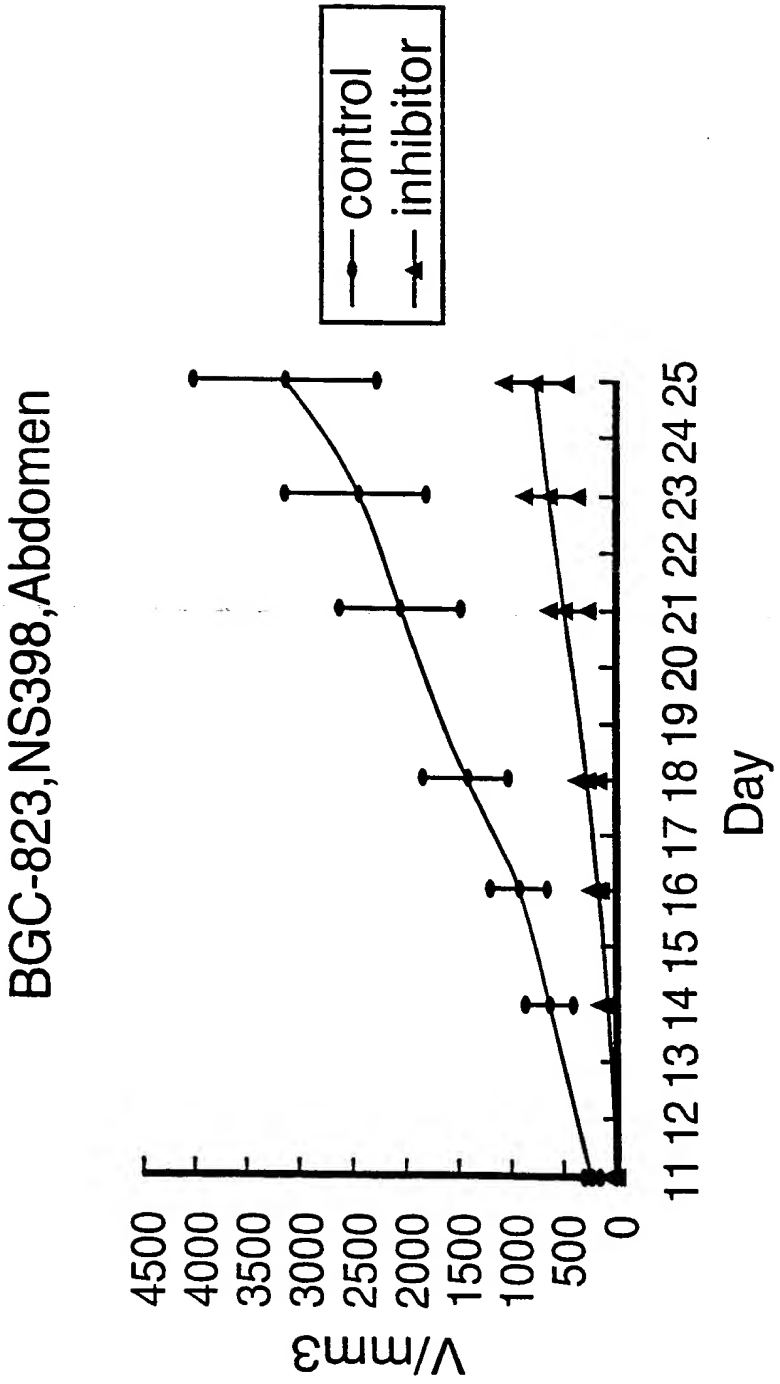
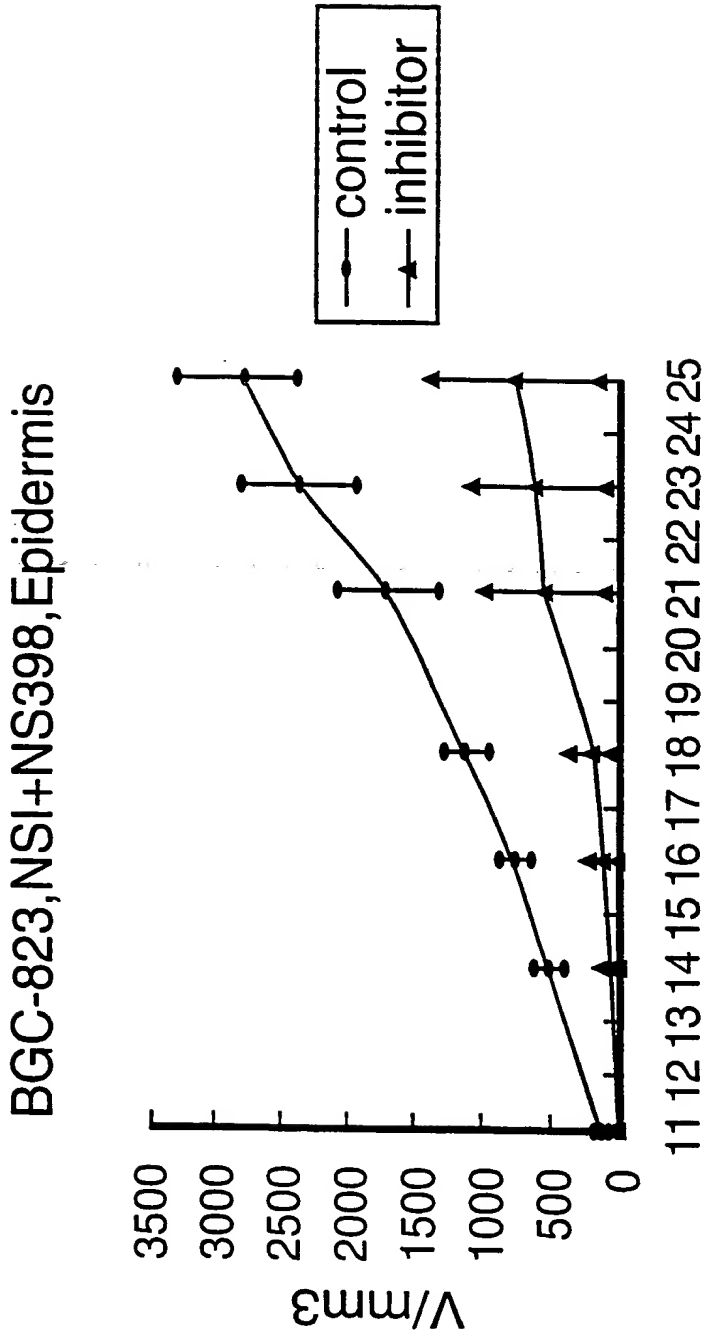

BGC-823,NS398,Abdomen
control
inhibitor
V/mm3
4500
4000
3500
3000
2500
2000
1500
1000
500
0
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Day

Figure 1


BGC-823,NSI+NS398,Epidermis
control
inhibitor
V/mm3
3500
3000
2500
2000
1500
1000
500
0
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Day

Figure 6

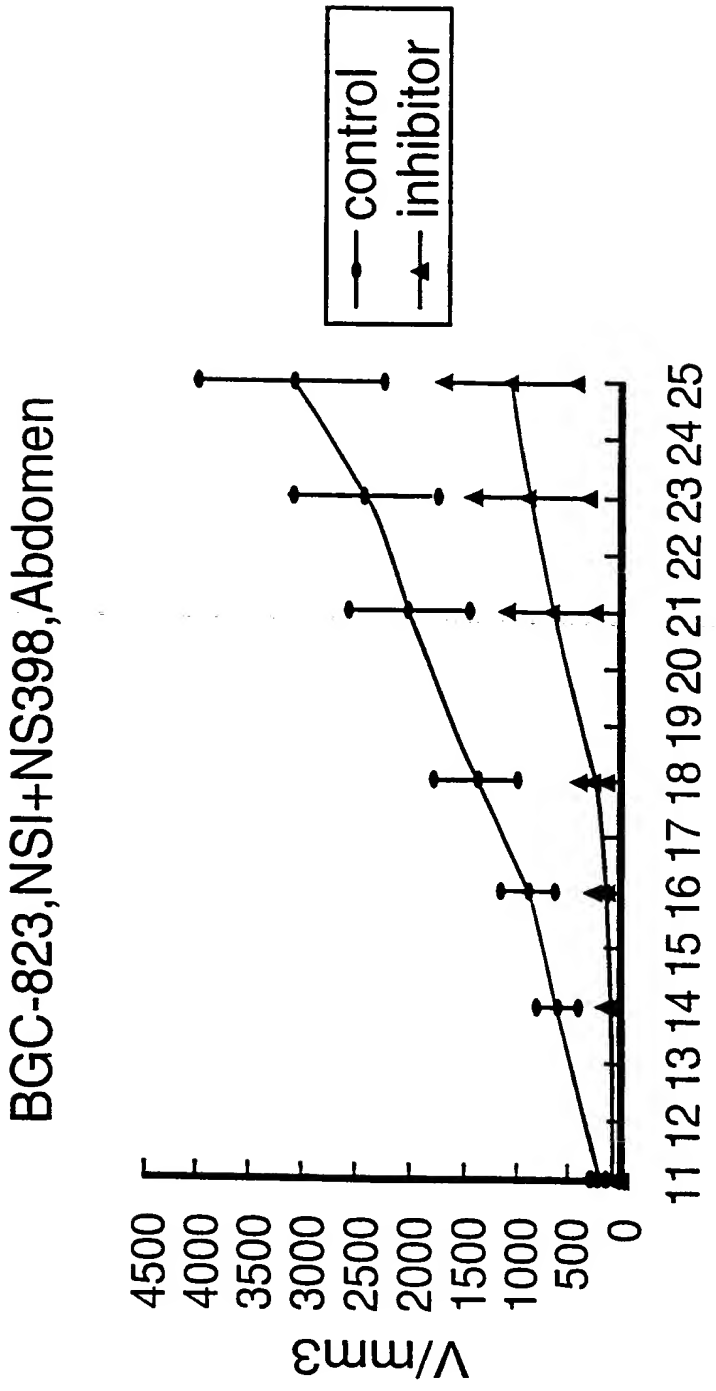
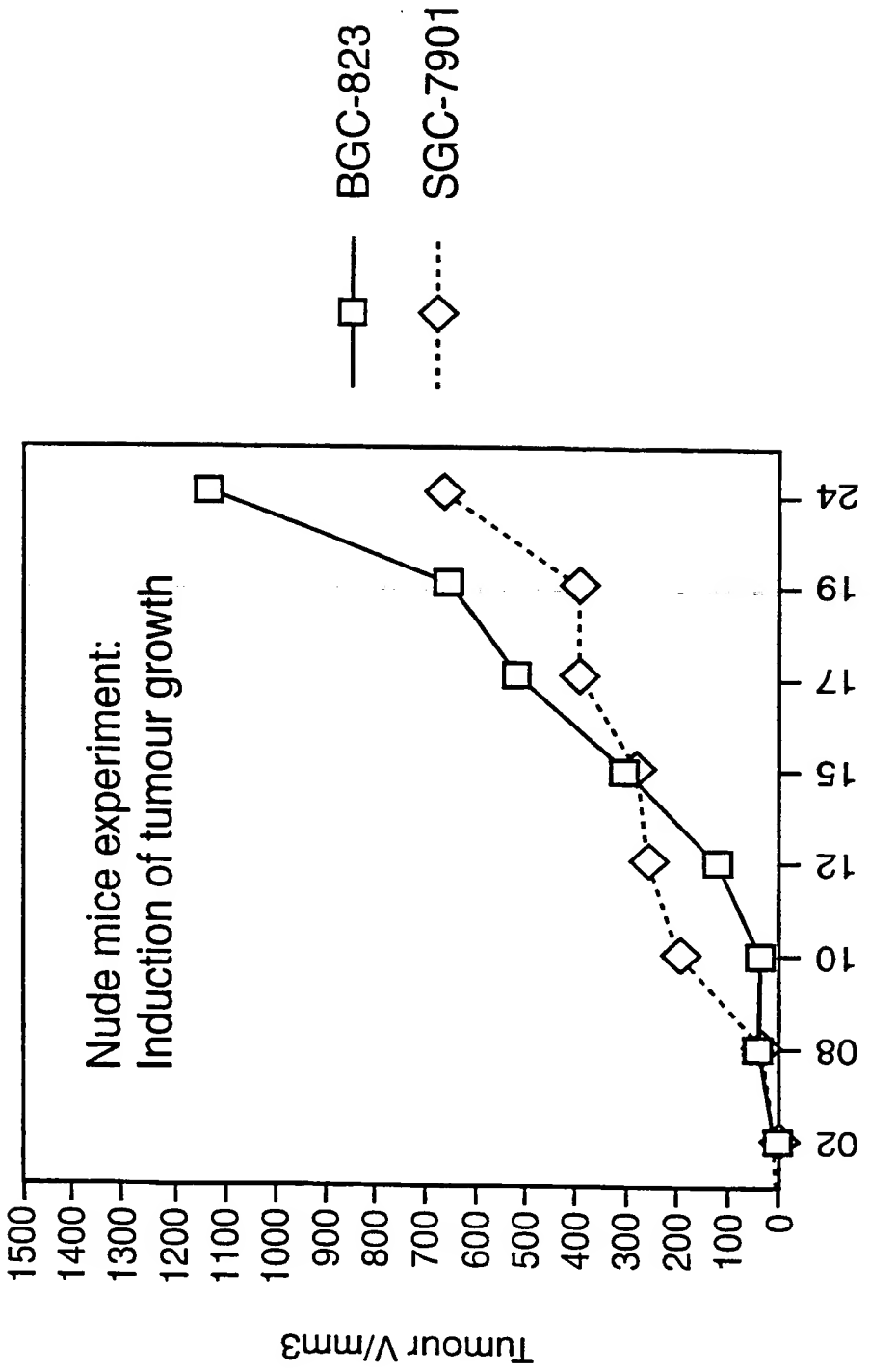

BGC-823,NSI+NS398,Abdomen
control
inhibitor
V/mm3
4500
4000
3500
3000
2500
2000
1500
1000
500
0
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Day

Figure 7


BGC-823
SGC-7901
Nude mice experiment:
Induction of tumour growth
Tumour V/mm3
0
100
200
300
400
500
600
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02
08
10
12
15
17
19
24

Figure 8

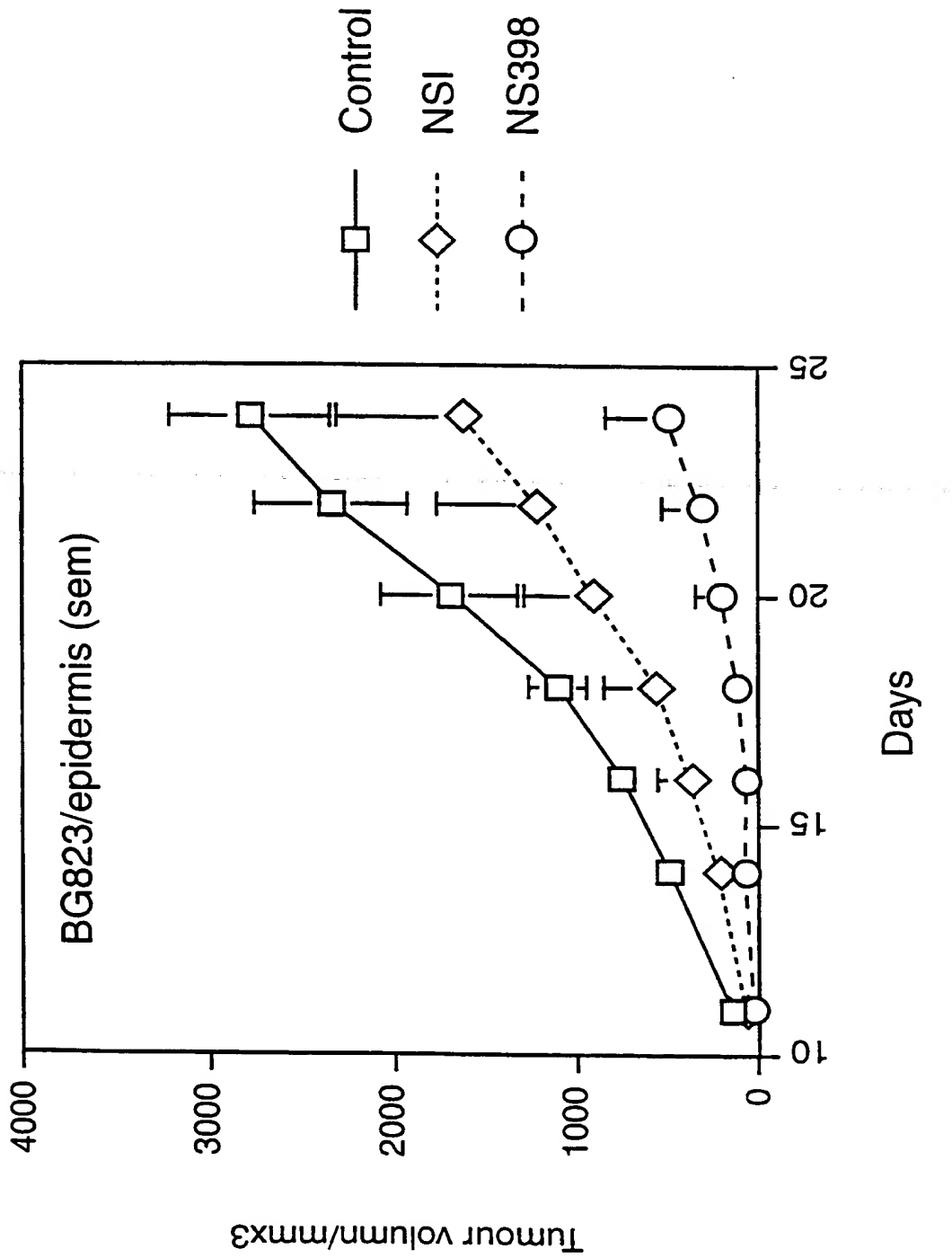
Control
NSI
NS398
BG823/epidermis (sem)
Tumour volumn/mmx3
4000
3000
2000
1000
0
10
15
20
25
Days

Figure 9

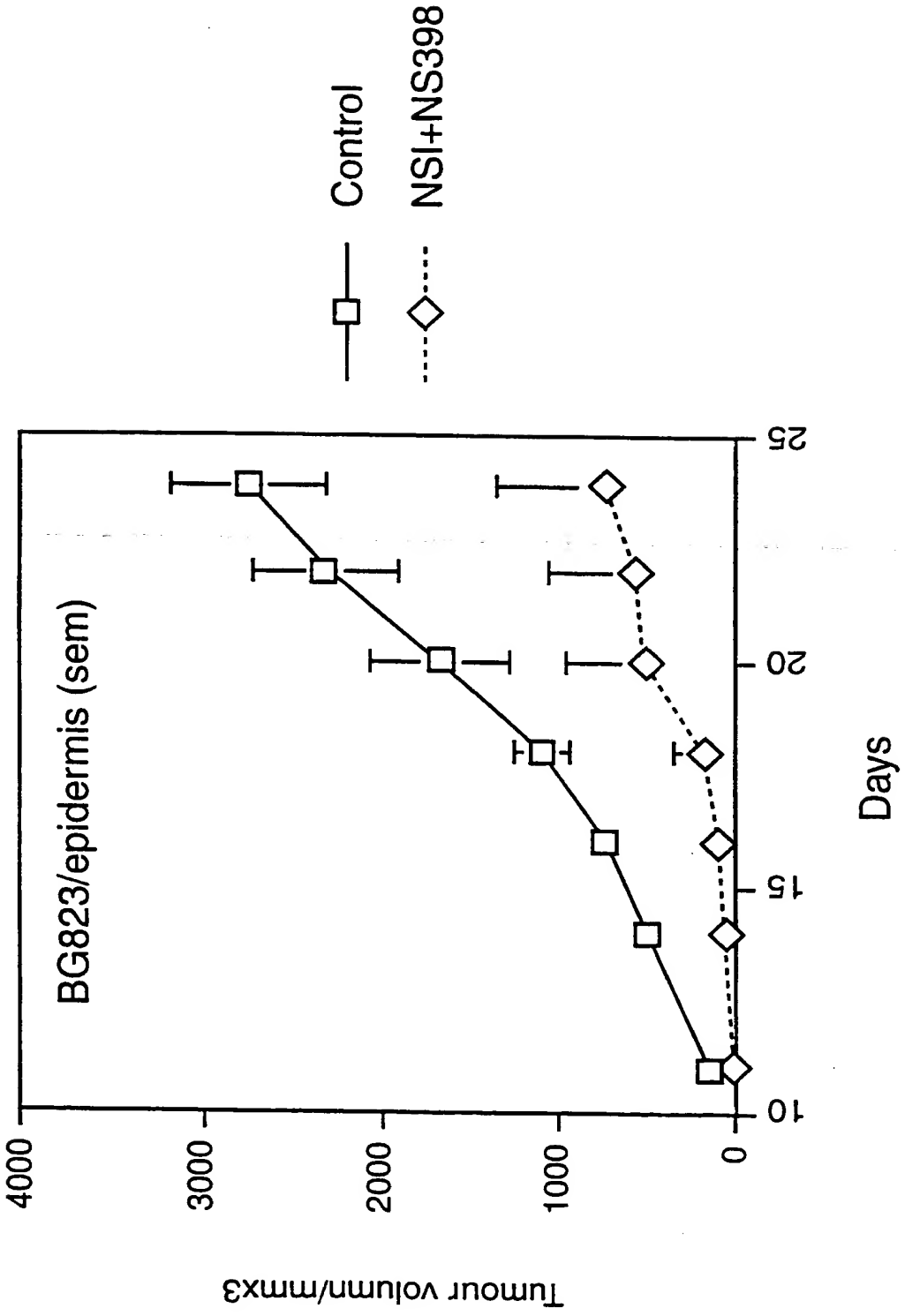
Control
NSI+NS398
BG823/epidermis (sem)
Tumour volume/mmx3
4000
3000
2000
1000
0
10
15
20
25
Days

Figure 10

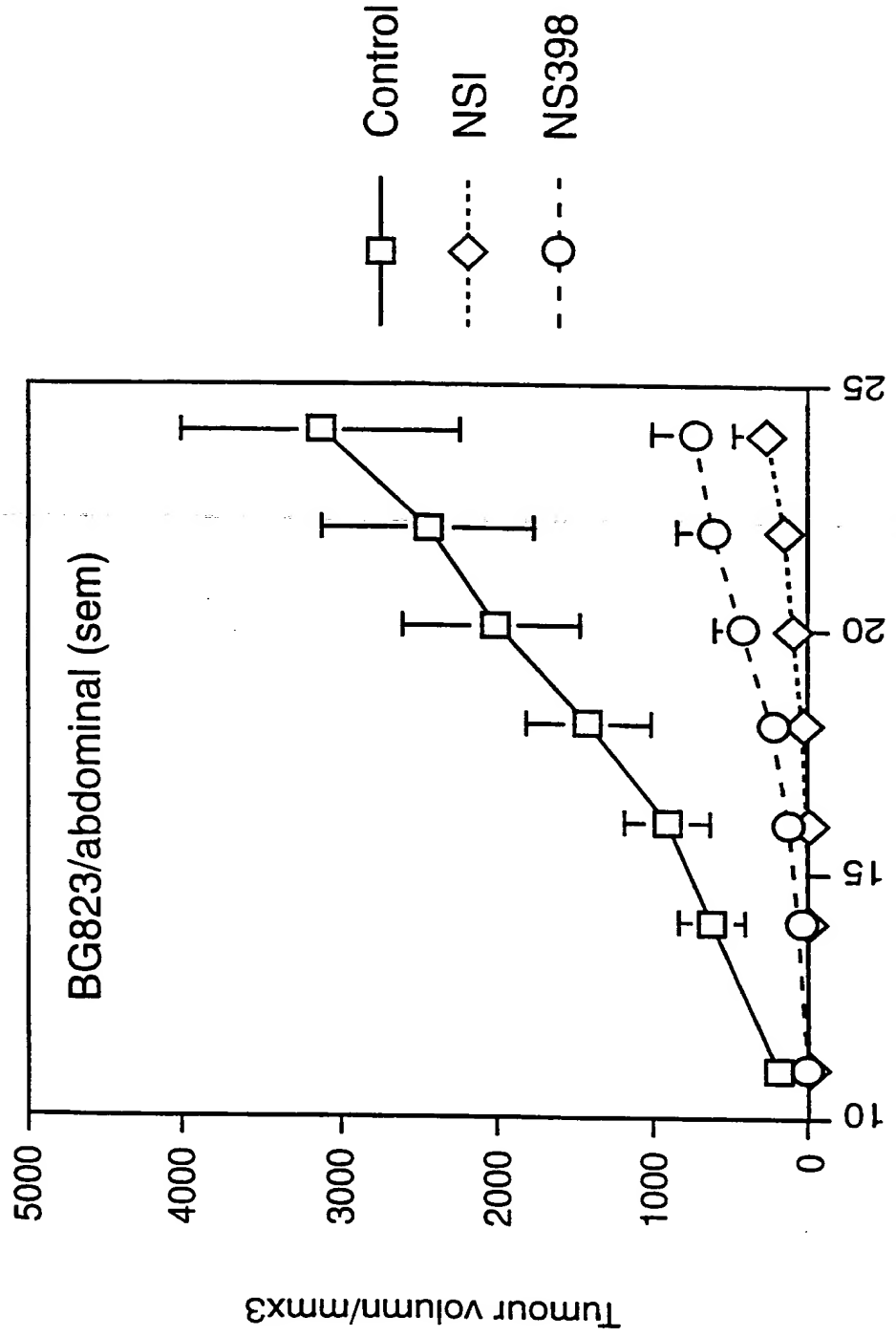
Control
NSI
NS398
BG823/abdominal (sem)
Tumour volumn/mmx3
5000
4000
3000
2000
1000
0
10
15
20
25
Days

Figure 11

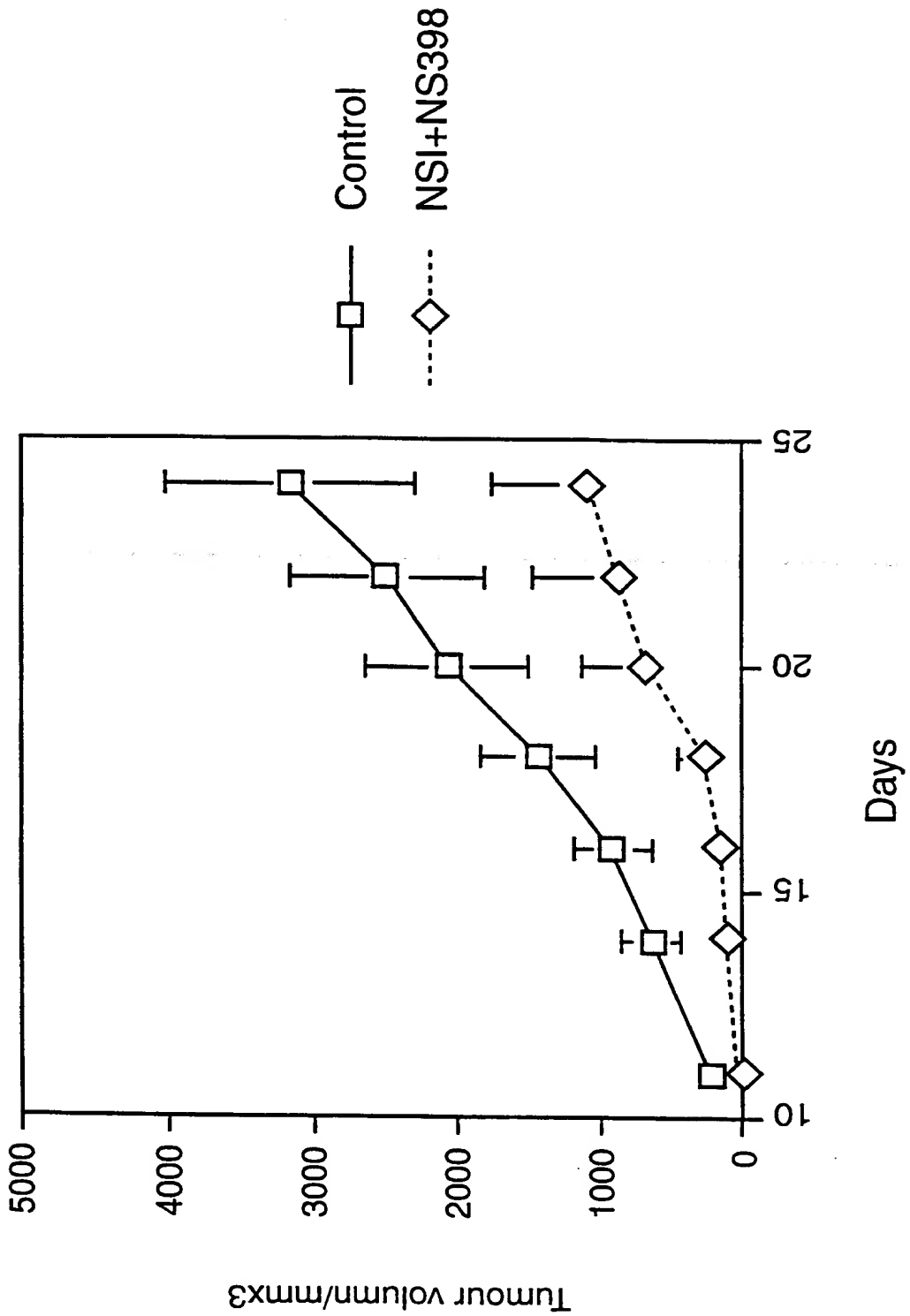
Control
NSI+NS398
Tumour volumn/mmx3
5000
4000
3000
2000
1000
0
10
15
20
25
Days

Figure 12

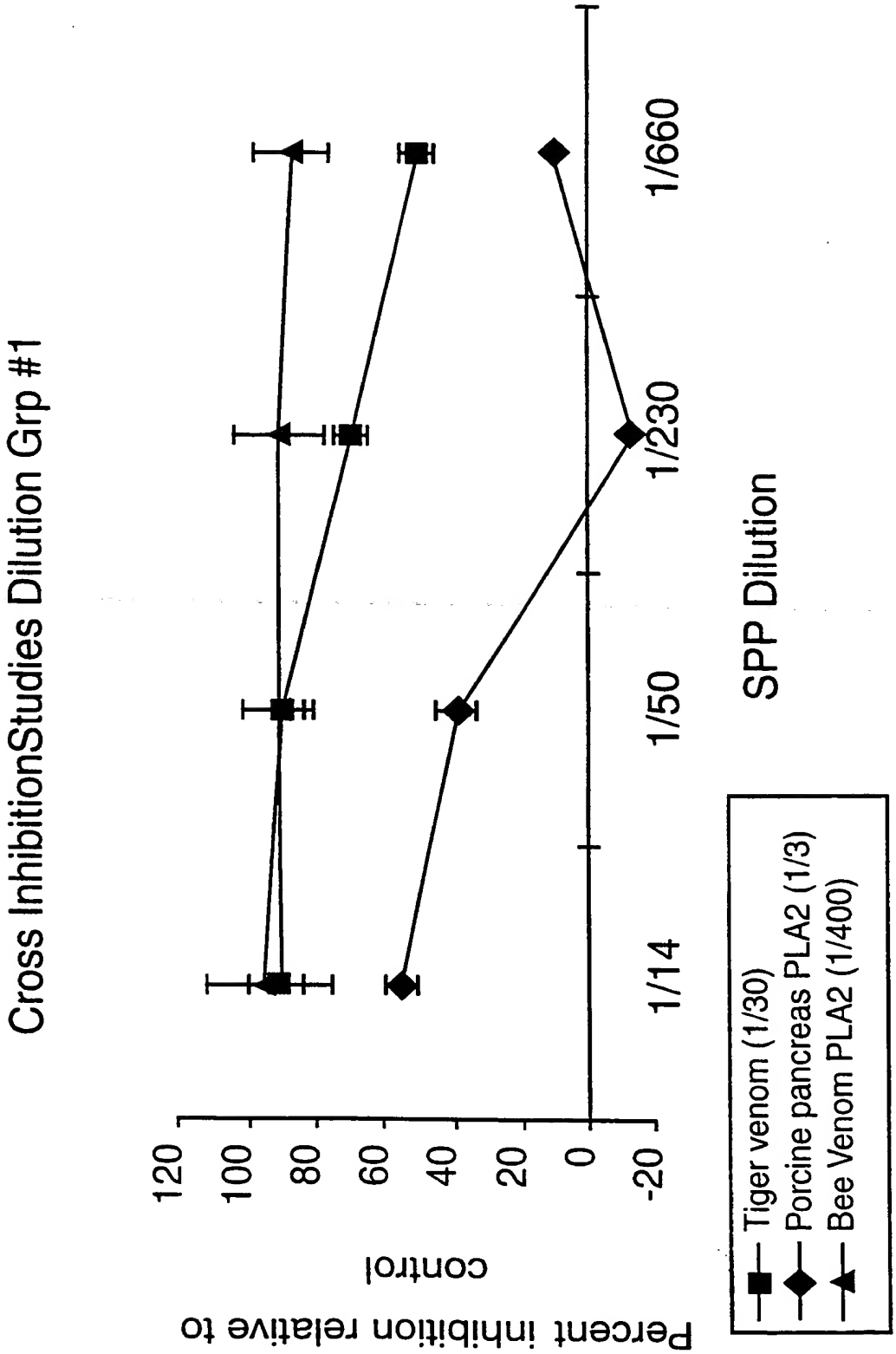
Cross InhibitionStudies Dilution Grp #1
Percent inhibition relative to control
120
100
80
60
40
20
0
-20
1/14
1/50
1/230
1/660
SPP Dilution
Tiger venom (1/30)
Porcine pancreas PLA2 (1/3)
Bee Venom PLA2 (1/400)

Figure 13a

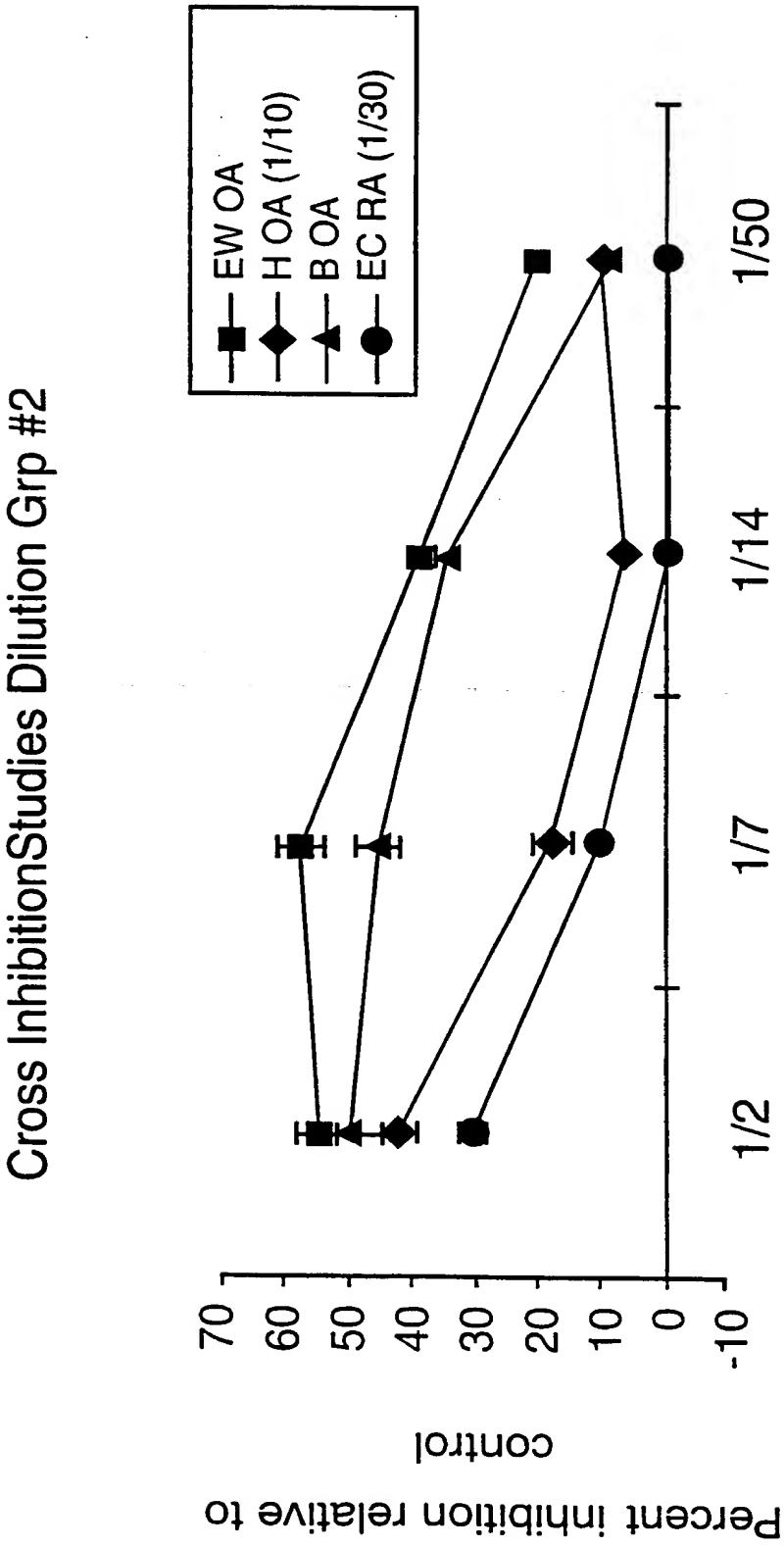
Cross InhibitionStudies Dilution Grp #2
EW OA
H OA (1/10)
B OA
EC RA (1/30)
Percent inhibition relative to control
70
60
50
40
30
20
10
0
-10
1/2
1/7
1/14
1/50
SPP Dilution

Figure 13b

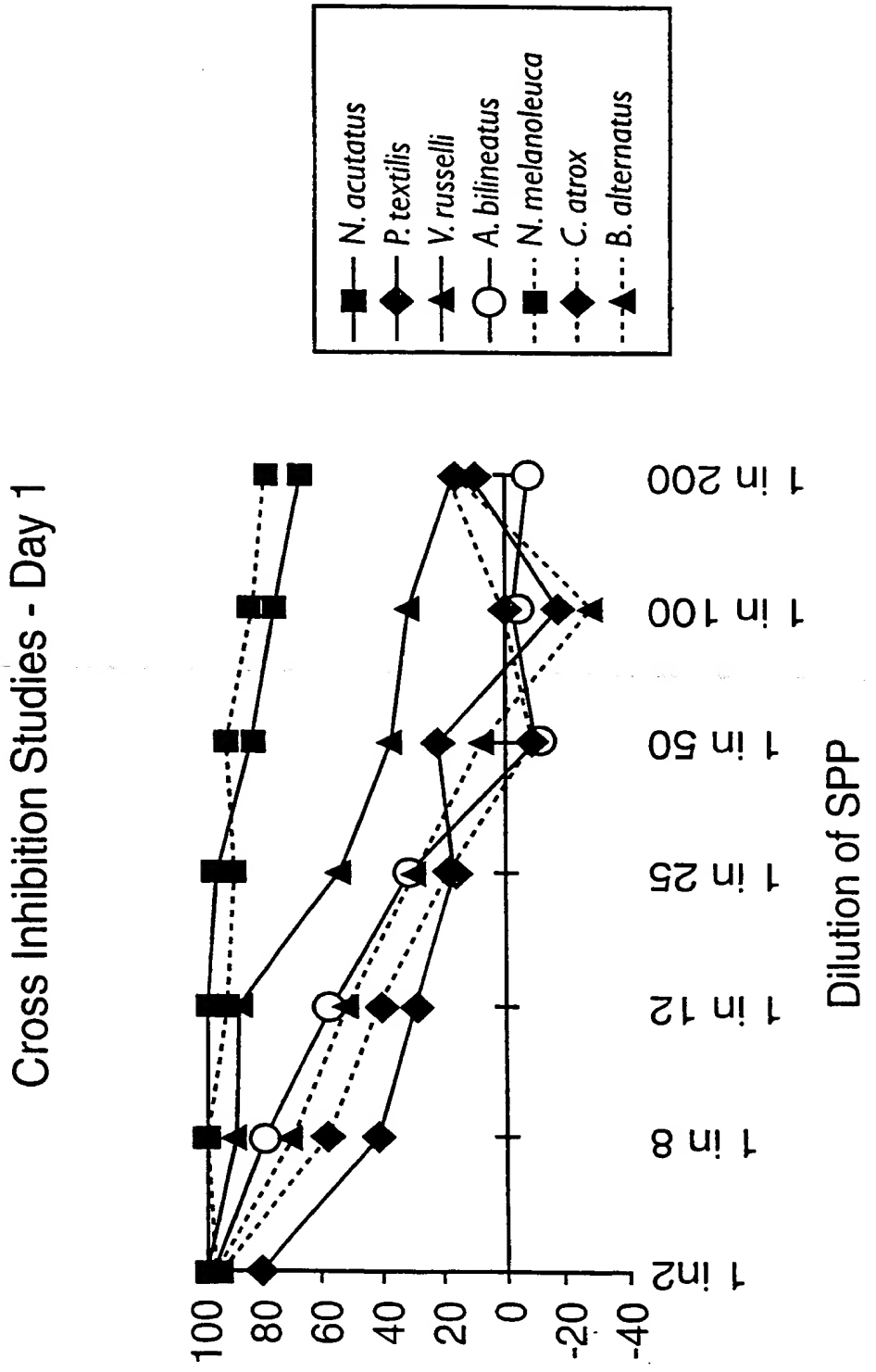
N. acutatus
P. textilis
V. russelli
A. bilineatus
N. melanoleuca
C. atrox
B. alternatus
Cross Inhibition Studies - Day 1
Percent inhibition relative to control
100
80
60
40
20
0
-20
-40
1 in2
1 in 8
1 in 12
1 in 25
1 in 50
1 in 100
1 in 200
Dilution of SPP

Figure 14a

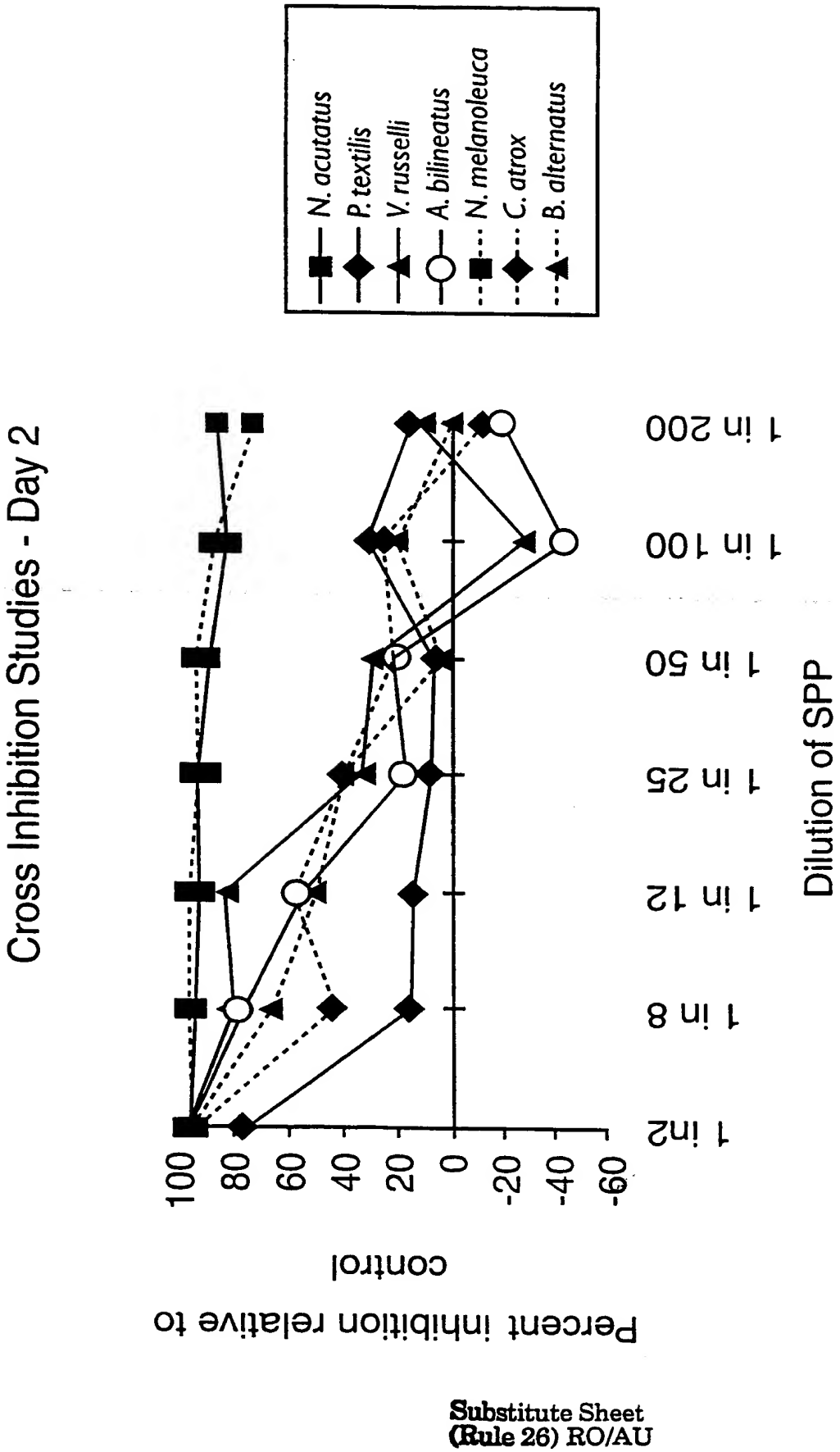
Cross Inhibition Studies - Day 2
N. acutatus
P. textilis
V. russelli
A. bilineatus
N. melanoleuca
C. atrox
B. alternatus
Percent inhibition relative to control
100
80
60
40
20
0
-20
-40
-60
1 in2
1 in 8
1 in 12
1 in 25
1 in 50
1 in 100
1 in 200
Dilution of SPP

Figure 14b

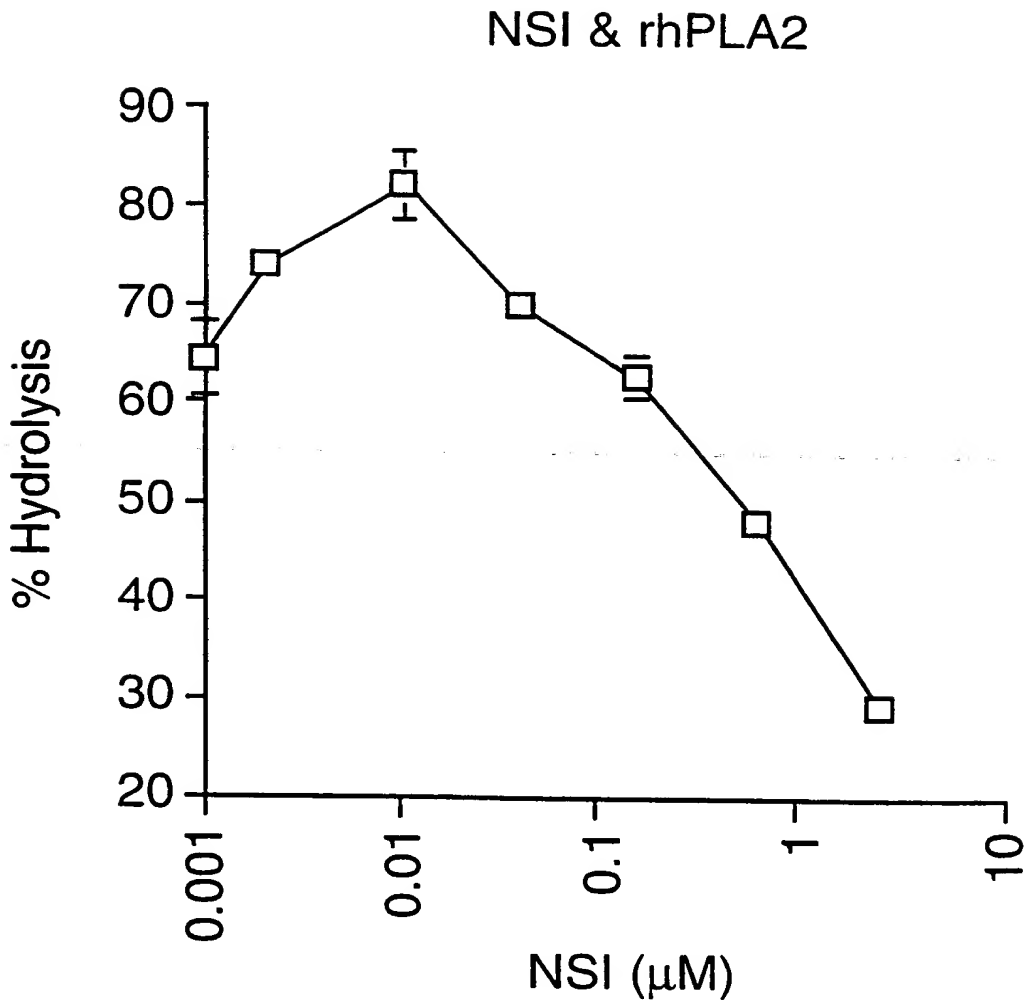
NSI & rhPLA2
% Hydrolysis
90
80
70
60
50
40
30
20
0.001
0.01
0.1
1
10
NSI (μM)

Figure 15

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (US only) BROADY, K. W. and TSENG, A. P. S.
(other than US) Analytica, Ltd

(ii) TITLE OF INVENTION: A METHOD OF TREATMENT

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: International PCT
(B) FILING DATE: 1999/11/12

(vii) PRIORITY DATA:
(A) APPLICATION NUMBER: 60/108254
(B) FILING DATE: 1998/11/12

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HUGHES, DR E JOHN L

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
(B) TELEFAX: +61 3 9254 2770
(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
 1               5                  10                  15

Gly Ser Cys His Ser Cys Glu Ile Cys His Asn Leu Gly Arg Asp Cys
            20                  25                  30

Glu Thr Glu Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
        35                  40                  45

Thr Val Leu Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile
    50                  55                  60

His Arg Asn Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp
65                  70                  75                  80

Ile Asn Ile Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys
                85                  90                  95

Asp Glu Ala Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser
            100                 105                 110

Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val
        115                 120                 125

Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys
    130                 135                 140

Ile Asn Leu Ala Gly Phe Arg Lys Glu Arg Phe Pro Gly Asp Ile Ala
145                 150                 155                 160

Tyr Asn Ile Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser
                165                 170                 175

Asn Arg Thr His Glu Glu Asp Arg Asn Gly Leu Ile Lys Val Glu Cys
            180                 185                 190
```

Thr Asp Ala Ser Lys Ile Thr Pro Ser Glu
195 200

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 202 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ile Ser Leu Gln Ile Ile Cys Phe Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys His Ser Cys Glu Ile Cys Arg Asn Phe Gly Lys Asp Cys
20 25 30

Glu Ser Glu Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
35 40 45

Thr Val Leu Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile
50 55 60

His Arg Asn Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp
65 70 75 80

Ile Asn Ile Gly His Asp Ser Tyr Val Arg Gly Arg Ile His Cys Cys
85 90 95

Asp Glu Glu Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Pro Ser
100 105 110

Leu Pro Asn Gly Tyr His Cys Pro Gly Ile Leu Gly Ala Phe Ser Val
115 120 125

Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys
130 135 140

Ile Asn Leu Ala Gly Phe Arg Lys Glu Arg Tyr Pro Val Asp Ile Ala
145 150 155 160

Tyr Asn Ile Thr Gly Cys Thr Ser Ser Cys Pro Glu Leu Lys Leu Ser
165 170 175

```
Asn Arg Thr His Ala Glu Arg Arg Asn Ala Leu Ile Thr Leu Asp Cys
            180                 185                 190

Thr Asp Ala Ser Lys Ile Ala Pro Ser Glu
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG AAA TCC CTA CAG ATC ATC TGT CCT CTT TTC GTT TTG GTA GCC AGA      48
Met Lys Ser Leu Gln Ile Ile Cys Pro Leu Phe Val Leu Val Ala Arg
  1               5                  10                  15

GGA AGC TGT CGC TCA TGT GAA ATT TGT CAC AAT TTT GGA AAA GAT TGC      96
Gly Ser Cys Arg Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys
            20                  25                  30

GAG AGT GAG GAG GCA GAG GAA TGT GCC TCT CCA GAA GAT CAA TGT GGC     144
Glu Ser Glu Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
        35                  40                  45

ACA GTG TTG CTG GAG ATT TCA TCA GCA CCT ATT TCC TTC CGA TCC ATT     192
Thr Val Leu Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile
    50                  55                  60

CAT AGG AAC TGT TTC TCA TCC AGC CTC TGC AAA CTT GAA CAC TTT GAT     240
His Arg Asn Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp
65                  70                  75                  80

ATA AAT ATT GGA CAT GAT TCC TAT GTG AGA GGA AGA ATC CAC TGT TGT     288
Ile Asn Ile Gly His Asp Ser Tyr Val Arg Gly Arg Ile His Cys Cys
                85                  90                  95
```

```
GAT GAA GAA AGG TGT GAA GGA CAG CAA TTT CCT GGA CTG CCC CTC TCC      336
Asp Glu Glu Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser
            100                 105                 110

TTT CCA AAT GGA TAC CAC TGC CCT GGC ATT CTT GGT GCA TTC TCA GTG      384
Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Leu Gly Ala Phe Ser Val
        115                 120                 125

GAC AGC TCT GAA CAT GAA GCT ATT TGC AGA GGA ACC GAA ACC AAA TGC      432
Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys
    130                 135                 140

ATT AAC CTT GCG GGA TTC AGA AAA GAA AGA TAT CCT GTA GAC ATC GCT      480
Ile Asn Leu Ala Gly Phe Arg Lys Glu Arg Tyr Pro Val Asp Ile Ala
145                 150                 155                 160

TAT AAT ATC AAA GGT TGC ACT TCT TCT TGT CCA GAA CTG AAG TTG AGC      528
Tyr Asn Ile Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Lys Leu Ser
                165                 170                 175

AAT AGA ACT CAC GAA GAA CGT AGA AAT GAT CTA ATA ACA CTT GAA TGT      576
Asn Arg Thr His Glu Glu Arg Arg Asn Asp Leu Ile Thr Leu Glu Cys
            180                 185                 190

ACA GAT GCC TCC AAA ATT ACA CCT TCC GAA TAA                          609
Thr Asp Ala Ser Lys Ile Thr Pro Ser Glu
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Xaa
1               5                  10                  15
```

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val
20 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Leu Ser Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Thr
1 5 10 15

Pro His Asn

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr Glu Val
1 5 10 15

Ile Arg

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Pro Val Thr Leu Gly Leu Ile
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Glu Cys Thr Glu His Leu Val Ser Cys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Ser Cys Glu Ile Cys His Asn Phe Gly Arg Asp Cys Gln Ser Asp
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly

20 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Ser Cys Glu Ile Cys His Asn Leu Gly Lys Asp Cys Glu Thr Glu
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
20 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ile Thr Pro Ser Glu
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Phe Asp Ile Asn Ile
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ile Asn Leu Ala Gly Phe
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Ser Lys Ile Thr Pro Ser Glu
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Tyr Pro Gly Asp Ile Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val
20 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Leu Ser Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Thr
1 5 10 15

Pro His Asn

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr Glu Val
1 5 10 15

Ile Arg

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ala Pro Val Thr Leu Gly Leu Ile
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Glu Cys Thr Glu His Leu Val Ser Cys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Phe Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gly Ser Glu Asn Gln Cys Lys Ser Ile Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Val Asn Pro Pro Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser
1 5 10 15

Ser Leu Glu Cys Thr Glu
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Phe Gly Leu Phe Phe Arg
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Glu Gly Gly
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Thr Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile

50 55 60

Gly His Asp Ser Phe Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Leu Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Arg Glu Arg Phe Pro Gly Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala
165 170 175

Ser Lys Asn Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Asp

1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Ala Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Lys
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Asn Arg Thr His
145 150 155 160

Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala Ser
165 170 175

Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

His	Ser	Cys	Glu	Ile	Cys	His	Asn	Phe	Gly	Lys	Asp	Cys	Glu	Gly	Gly
1				5					10						15
Val	Thr	Glu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu
			20					25					30		
Leu	Glu	Val	Ser	Thr	Ala	Pro	Ile	Ser	Thr	Arg	Thr	Ile	His	Arg	Asn
		35					40					45			
Cys	Phe	Ser	Ser	Ser	Leu	Cys	Lys	Leu	Glu	Arg	Phe	Asp	Ile	Asn	Ile
	50					55					60				
Gly	His	Asp	Ser	Tyr	Met	Arg	Gly	Arg	Ile	His	Cys	Cys	Asp	Glu	Ala
65					70					75					80
Arg	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn
				85					90					95	
Gly	Tyr	His	Cys	Pro	Gly	Ile	Leu	Gly	Leu	Phe	Ser	Val	Asp	Ser	Ser
			100					105					110		
Glu	His	Glu	Ala	Ile	Cys	Arg	Gly	Ser	Glu	Thr	Lys	Cys	Ile	Lys	Ile
		115					120					125			
Ala	Gly	Phe	Arg	Arg	Glu	Arg	Tyr	Pro	Ile	Asp	Ile	Ala	Tyr	Asn	Ile
	130					135					140				
Lys	Gly	Cys	Thr	Ser	Ser	Cys	Pro	Glu	Leu	Arg	Leu	Ser	Asn	Arg	Thr
145					150					155					160
His	Glu	Glu	His	Arg	Asn	Asp	Leu	Ile	Lys	Val	Glu	Cys	Thr	Asp	Ala
				165					170					175	

Ser Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly His Lys Val Asn Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Leu Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGA GACAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGATGG AGGTTTCAAC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTTTTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGAATTC TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAGAG AAAGATTTCC TGGAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ATAGAAATGA G 501

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCC AGAGTGACGA GACAGAGGAA 60

TGTGCCTCTG CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAAGTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TATAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAA TAGAACTCAC 480

GAAGAACATA GAAATGATCT A 501

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGT GACAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGGTTTCAAC AGCACCTATT 120

TCCACCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATATG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

AGTGAAACCA AATGCATTAA AATTGCGGGA TTCAGAAGAG AAAGATATCC TATAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ATAGAAATGA T 501

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 825 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CTTGAGTGTG AGATTTGTAT CGGGCTGGGC CTGGAATGTA ACACCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACAGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA CACAAAGTCA ACCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCTGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAAGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA AACAACATAC TCCAAAACAG CCAAAACAGC CAAAACAGCA 540

CTACATACTC CTAACCGTAT GCACAACAAC CAAAAATGAA ATCCCTACAG ATCATCTGTC 600

TTCTTTTCGT TTTGGTAGCC AGAGGAAGCT GTCAAAAATG AAATCCCTAC AGATCATCTG 660

TCTTCTTTTC GTTTTGGTAG CCAGAGGAAG CTGTCAAAAA TGAAATCCCT ACAGATCATC 720

TGTCTTCTTT TCGTTTTGGT AGCCAGAGGA AGCTGTACTA CAACCTAAAT GAAGTCCCTC 780

TTATTCTGTT GCCTCTTTGG CACTTTCTTA GCTACAGGCA TGTGT 825

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57